NEUROPHARMACOLOGICAL ACTIVITY OF *Dolichandrone atrovirens* (*roth*) IN SWISS ALBINO MICE

Dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY, CHENNAI.

In partial fulfillment of the requirement for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

By

(Reg No: 261525351)

Under the guidance of

Dr.P.Thirupathy Kumaresan, M.Pharm., Ph.D.,

Professor

DEPARTMENT OF PHARMACOLOGY

ARULMIGU KALASALINGAM COLLEGE OF PHARMACY

ANAND NAGAR, KRISHNANKOIL-626126

MAY-2017

Dr. N.VENKATESHAN , M.Pharm., Ph.D.,

Principal and Professor,

Department of Pharmaceutical Chemistry,

Arulmigu Kalasalingam College of Pharmacy,

Anand Nagar, Krishnankoil.

CERTIFICATE

This is to certify that the investigation described in this dissertation entitled **NEUROPHARMACOLOGICAL ACTIVITY OF** *Dolichandrone atrovirens (roth)* **IN SWISS ALBINO MICE** Submitted by Reg No: 261525351 was carried out in the Department of Pharmacology, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil-626126, Which is affiliated to The Tamilnadu Dr.M.G.R.Medical University, Chennai, Under the Guidance of **Dr.P.Thirupathy Kumaresan, M.Pharm., Ph.D.,** Professor, Department of Pharmacology for the partial fulfillment of the degree of MASTER OF PHARMACY in PHARMACOLOGY, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil-626126.

Place: Anand Nagar

Date :

Dr.N.VENKATESHAN,M.Pharm.,Ph.D.,

Principal / Professor

Dr. P.Thirupathy Kumaresan, M.Pharm., Ph.D.,

Professor & H.O.D of Pharmacology,

Arulmigu Kalsalingam College of Pharmacy,

Anand Nagar, Krishnankoil.

CERTIFICATE

This is to certify that the investigation described in this dissertation entitled **NEUROPHARMACOLOGICAL ACTIVITY OF** *Dolichandrone atrovirens (roth)* **IN SWISS ALBINO MICE** Submitted by Reg No: 261525351 was carried out in the Department of Pharmacology, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil-626126,which is affiliated to The Tamilnadu Dr.M.G.R. Medical University, Chennai, Under the supervision and guidance of Dr.P.Thirupathy Kumaresan, M.Pharm, Ph.D., Professor, Department of Pharmacology for the partial fulfillment of degree of MASTER OF PHARMACY in PHARMACOLOGY.

Place:Anand Nagar **Dr.P.Thirupathy Kumaresan,M.Pharm.,Ph.D.,**

Date:

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **NEUROPHARMACOLOGICAL ACTIVITY OF** *Dolichandrone atrovirens (roth)* **IN SWISS ALBINO MICE** submitted by Reg.No:261525351, was evaluated for the partial fulfillment of the requirements for the award of the degree of **MASTER OF PHARMACY** in **PHARMACOLOGY,** The Tamilnadu Dr.M.G.R. Medical University, Chennai.

Date :

Centre: Arulmigu Kalasalingam College Of Pharmacy

Krishnankoil

Examiners

1.

2.

THIS IS DEDICATED

TO

GOD AND MY

FAMILY

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

"**You will meet more angels on a winding path than on a straight one"**

Research is formalized curiosity. It is poking and prying with a purpose. Working on a research project needs guidance, support and encouragement. Getting such help is only by the grace of God.

First and foremost, I would like to thank the almighty God Jesus for giving me strength in my weakness and guiding me through all my darkness and taught the way in a difficult part of life. The completion of this project is not only fulfillment of my dream but also in fulfillment of the dream of my parents who have taken lots of pain in my making.

I hereby take this opportunity to acknowledge all those who have helped me in the completion of this dissertation work.

I would like to express our thanks to the founder of our institution "**Kalvivallal" Thiru. T.Kalasalingam,B.com** for providing us required facilities for extending a rich.And also I convey thank to "**Ilaiya vallal" Dr.K.Sridharan, Ph.D.,** Dynamic directors **Dr.S.Shasi anand, Ph.D., Mr.S.Arjun Kalasalingam, M.S.,** and management of our Institution for providing us necessary infrastructure.

It is an honour to pay my respect and heartfelt thanks to our most respected Principal **Dr. N.Venkateshan, M.Pharm., Ph.D.,** who gave me the opportunity to do this project in our Institution. And I thank for his most valued suggestions during the course of study and providing Permission to utilize the facilities available in the institute for my project work.

I respectfully acknowledge to our **Research Director Dr.V.Lavakumar, M.Pharm, Ph.D.,** for providing suggestions, encouragements during the project. Especially for his patience and immense knowledge. His constants quest for knowledge and strive for excellence will always remain a source of inspiration to me.

It give me immense pleasure to express my deepest thanks, heartfelt, indebtedness and respectful Guide **Dr.P.Thirupathy Kumaresan M.Pharm., Ph.D.,** for his encouragement and guidance during the course of the project and special thank for providing suggestions during the project.

I deem it a great pleasure to place on record my deep sense of gratitude to **Dr.N.R.Livingston Raja M.Pharm., Ph.D.,** for his guidance and constant encouragement during the course of study. His guidance helped me in all the time of research and writing of this dissertation.

I express my special thanks to **Mr. M.Santhana Kumar, M.Pharm,** For providing much of stimuli in the form of suggestions and guidance were of enormous support for me during my entire research work. He was a good mentor to answer any question regarding research.

I would like to thank, our faculty member **Mr.V.Sivakumar, M.Pharm.,(PhD)**, **Dr.J.JeyaAnanthi., M.Pharm., Ph.D.,** Department of Pharmaceutics, **Ms.P.Anitha**, **M.Pharm,** Department of Pharmacognosy, Especially I would thank for their kindness guidance and clearing the doubt at any time and for their generosity in extending help.

I am deeply thankful to the **Mrs.V.Karpagavali MCA**,**M.Phil,B.Ed.,** for providing permission to use the Computer lab and clearing doubts while typing work at any time with smiling face.

I also convey my thanks also all the Lab assistants of our Institution, especially **Mr.R.Ramanantham, Mr.C.Ganesan**, **Mr.P.LaxmanaGuruSamy**, **Mr.V.Siva Gurusamy, T.Senthamaraikannan, DMLT.**

My sincere thanks to **Dr.D.Stephen** (Head, Department of Botany, American college, Madurai) for his help in identification & authentication of the plant.

A warm thank to all my friends who have lent a hand to complete this dissertation.Especially I thank **D.Sivakumar, J.Arunpandiyan, H.Ganesh, M.Thiruppathi, P.Vijay nagendiran, R.Nivetha** and my junior friends in developing the project and friends who have willingly helped me out with their abilities for completing the project.

I would never forget all the beautiful moments given by my **Rizia** and full support of my **sister.**I would thank for their kind care of me and supportive by their own way.

Above all without **my parents** this accomplishment in my life would never have Begun to take form; to them, I give special thanks. I want to express my respect to my father **V.Rajan Durai,** Associated Capsules Ltd, Mumbai. The pursuit of this degree would not have Been possible without his encouragement in the great Pharmacy field,

financial support whenever am asking without saying the word “NO”. I really appreciate unreserved love and support from my mother **R.Chandra,** who spent a very long time praying for me. She gave me a considerable support and advice to be calm and to ensure my progress.I am Grateful to have a such a parent and my brothers and sisters. My humble thanks to all the **mentors, well-wishers, near and dear ones** who helped me during my course of study.

Thank you all

Arulmigu Kalasalingam College of Pharmacy

Anand Nagar, Krishnankoil - 626 126, Srivilliputtur (Via), Virudhunagar Dist.,Tamil Nadu, India.
Phone : 04563-289006
e-mail : akcpprl@yahoo.com ★ www.akcp.ac.in

"Kalvivallal"
T.Kalasalingam, B.Com.,
Founder Chairman & Correspondent

"Ilayavallal"
Dr.K.Sridharan, M.Com., MBA., Ph.D.,
Secretary

Dr.N.Venkateshan, M.Pharm., Ph.D.,
Principal

CERTIFICATE

This is to certify that the project title **Neuropharmacological Evaluation of *Dolichandrone atrovirens (Roth) k.Schum in Swiss albino mice*** has been approved by the Institutional Animal Ethical Committee.

IAEC approval No AKCP/IAEC/011/16-17 in date of 22.10.2016

Student Name: BABY ROSELIN.R **Reg.No:261525351**

IAEC Chairperson

Dr. D. Stephen
Professor
Department of Botany

The American College
Madurai-2
Mobile no: 9944792299

Authentification Certificate

This is to certify that the plant specimen brought to me by R.Baby Roselin, Final year M.Pharm (2016-2017), **Department of Pharmacology, Arulmigu Kalasalingam College Of Pharmacy,** Krishnankoil, Srivilliputhur, has been identified as ***Dolichandrone atrovirens*** belonging to the family **Bignoniaceae** .

Date: 10.08.2016

Dr. D. Stephen

Dr. D. STEPHEN, Ph.D.,
ASST. PROFESSOR IN BOTANY
THE AMERICAN COLLEGE
MADURAI - 625 002
TAMILNADU-INDIA

CONTENTS

TITLE OF CONTENT

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

“There are no incurable diseases - only the lack of will.

There are no worthless herbs - only the lack of knowledge”. - Avicenna

“Genesis 1:29 And God said , Behold , I have given you every herb bearing seed,which is upon the face of all the earth,and every tree, in the which is the fruit of a tree yielding seed;to you it shall be for meat.”According to WHO 50% Man’s dependence on plants from his life began since human race [1]. No plant on this earth is completely worthless.The man depends directly or indirectly upon plants for their very basic needs of survival , food, fodder, fuel, fiber, fertilizer, timber, liquor and medicines [2,3]. Plants have been used for medicinal purposes long before prehistoric period.In the ancient time human knowledge found the absence of some food forms the base for the development of any disease,they were trying to use the same food material for curing that particular disease and they got success in that work.This motivates the plant researchers to use different plants,plant parts for different disease.

Besides, some plants are important source of nutrition and as a result of that these plants suggested for their therapeutic values. Herbs are alternative medicines for the treatment of various diseases due to their assumed acceptability, effectiveness, affordability, safety and low cost compared to conventional medicines. There is also a promising increase in the utilization of herbal formulations by the public because of the strong belief that these products are natural; hence, they are safe for the healing of ailments.

Alternative Medicine [4]

These days the term “Alternative Medicine” became very common in western culture, it focuses on the initiative of using the plants for medicinal purpose. But the current belief that medicines which come in capsules or pills are the only medicines that we belief and use. Even so most of these pills and capsules we take and use throughout our day by day life came from plants. Medicinal plants often used as raw materials for extraction of active ingredients which used in the synthesis of different

drugs. Like in case of laxatives, blood thinners, antibiotics and antimalarial medications, contain ingredients from plants. Moreover the active ingredients of Taxol, vincristine, and morphine isolated from foxglove, periwinkle, yew, and opium poppy, correspondingly.

Characteristics of Medicinal Plants [5]

Medicinal plants have many characteristics when used as a treatment, as follow:

Synergies medicine - The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

Support of official medicine - In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

Preventive medicine - It has been proven that the component of the plants is also characterizes by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present ,i.e., reduce the side effect of synthetic treatment.

Ancient Unani manuscripts Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as a medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically.

Traditional systems of medicine continue to be widely practised on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of a large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal)

medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practised in India.

Organizations like World Health Organization (WHO) and United Nations Children's Educational Fund (UNICEF) are very much interested in plants to be used for the treatment of various diseases of children. Plants and plant based drug are relatively less toxic and have acceptable side effects. It is hence important to bring the use of the remedies into an existing framework or rational scientific use of medicines.[6]

As per data available over three-quarters of the world population relies mainly on plants and plant extracts for their health care needs. More than 30% of the entire plant species, at one time or other were used for medicinal purposes. It has been estimated, that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as India and China, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine.

Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in link with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes.

Our traditional system of medicine of medicine siddha categorized nearly 5000 plant species and their usage.Later on the allopathic system of medicine comes to force and dominate the siddha and due to the fast relieving nature it reached the world as quickly and diminished the usage of plant medicine as maximum.But allopathy system cannot provide ultimate solution to some disease,and also their side effect in particularly the long term therapy, limits their usage still the plant medicine is recommended and used in such cases.This suggests the plant medicine to researchers as and scientific world as alternate to allopathy system of medicine.The world health organization also recognize and motivate the plant researchers and Centre,hence the plant medicine now considered being an alternative system of medicine Screening for the pharmacological

activity or biological activity is the first step in the research of the new drug obtained from an herbal source. The success is directly dependant on the correctness of identification of the plant source. So, identification and authentication is important in research of the drugs. All the experiments and subsequent analysis are done after identification of the plant by Botanist. Plant derived drugs are also used ins the developed countries like USA, Canada ,etc. For example, in USA 25% of all prescriptions dispensed are the plant extracts or the active compounds obtained from higher plants.

Even usage of plants are known,since plant species consists of mixture of compound,isolating the single compound and identifying the component is responsible for that particular activity is a major question in front of plant researcher and also it is very difficult to say only these are all the compounds available from particular plant.Nowadays due to the development of science and technology such as chromatographic technique and spectroscopical technique it is possible to isolate almost all the components of plant and characterize them.Isolation and characterization are very important to improve the effectiveness,minimizing the dose and onset of action.

Now this study is considered as a separate discipline called “Phytochemistry” defined as a branch of science somewhere in between natural product organic chemistry and plant biochemistry concerned with organic chemistry and plant biochemistry concerned with organic substances accumulated by plants and deals with the chemical structure of these substances,their biosynthesis,turnover,metabolism,their natural distribution and their biological function.(4)

Since detecting the compound responsible for the particular activity,isolating,characterizing the compound and monitoring the activity is of prime importance, the basic requirement needed for the medicinal world, it is the duty of the chemist to do these works**.**

”All that man needs for health and healing has been provided by God in nature,the challenge of science is to find it”.

Hope of Medicinal Plants

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies.[7]

As our lifestyle is now getting so advanced, we are moving away from nature.While we cannot escape from nature because we are the part of nature. As herbs are natural products they are free from side effects, they are comparatively safe, eco-friendly and locally available. Traditionally , there are lot of herbs used for the ailments related to different seasons. There is a need to promote them to save the human lives.These herbal products are today are the symbol of safety in contrast to the synthetic drugs, that are regarded as unsafe to human being and the environment. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Therefore, it is a very important point to encourage researchers and clinicians to work hard in order to clarify their role in the treatment of disease,and how can be used to produce or synthesis more effective drugs.

Their utility in allevatiating common problems with degenerative diseases and the ageing process is being promoted as a major factor for the popularity of this class of products. It has been found that, between 20,000 to 55,000 species of plants have been used globally, of which only a small portion has been investigated for therapeutic purposes. Among those that have been investigated are plant species that produces important drugs such as quinine, reserpine, tubocurarine, vincristine, vinblastine, pilocarpine, atropine, morphine, and cocaine, taxol, to mention a few. Overall, only 15-20% of terrestrial plants have been evaluated for pharmaceutical potentials.

Globally, there is a positive trend towards health, integrative sciences, systems biology approaches in drug discovery and therapeutics that has remained one of the unique features of Ayurveda. A golden triangle consisting of ayurveda, modern medicine and science will converge to form a real discovery engine that can result in newer, safer, cheaper and effective therapies.

CHAPTER II
REVIEW
OF LITERATURE

CHAPTER II

2 .1.Introduction

Mental, neurological and behavioral disorders are familiar to all countries and cause vast suffering. People with these disorders are often subjected to social segregation, poor eminence of life, and improved mortality. These disorders are the cause of staggering economic and social costs. Habituation, dependence and the resulting potential for addiction are the greater disadvantages of the modern synthetic psychopharmacological agents. The sudden discontinuation of long-term therapy with these drugs leads to serious withdrawal symptoms. Therefore, modern society is now cautiously discovering traditional herbal medicines, particularly the current studies for even better properties than the conventional medicines.

Introduction:

Neuropharmacology is the study of how drugs affect cellular function in the nervous system. Neuropharmacology is a very broad region of science that encompasses many aspects of the nervous system from single neuron manipulation to entire areas of the brain, spinal cord, and peripheral nerves. There are two main branches in neuropharmacology one is behavioural another one is molecular neuropharmacology.[8]

1. Behavioural neuropharmacology focuses on the study of how drugs affect human behaviour (neuropsychopharmacology), including the study of how drug dependence and addiction affect the human brain.

2. Molecular neuropharmacology involves the study of neurons and their neurochemical communications with the overall goal of developing drugs that have helpful effects on neurological function.

Both of these fields are closely connected since both are concerned with the interactions of neurotransmitters, neuropeptides, neurohormones, neuromodulators,

enzymes, second messengers, co-transporters, ion channels, and receptor proteins in the central and peripheral nervous systems. Abnormalities in the brain, spinal cord, or along the body's several nerves can create a wide range of symptoms such as paralysis, weakness, loss of harmonization, or even seizures. The nervous system, usually protected by the chemical composition of the blood-brain barrier, can be extremely vulnerable to damage if the protecting membranes are penetrated. Studying these interactions, researchers are developing drugs to treat many different neurological disorders including pain, neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Epilepsy, Meningitis, ADHD, psychological disorders, addiction, and many others.

In 2006, the World Health Organization (WHO) estimated that as many as one billion citizens worldwide suffer from several neurological disorder. One in four people in the world will be affected by mental or neurological disorders at some point in their lives. The report, Neurological disorders: Public health challenges, reveals that of the one billion people affected worldwide, 50 million bear from epilepsy and 24 million from Alzheimer and other dementias. Neurological disorders influence citizens in all countries, irrespective of age, sex, education or profits. An estimated 6.8 million people die every year as a result of neurological disorders.

Causes of neurological Disorder:[9]

The causes of such dysfunction can be quite diverse. Both the spinal cord and brain are insulated by numerous membranes that can be vulnerable force and pressure. The peripheral nerves located deep under the skin can also be vulnerable to damage. Neurological disorders can affect an entire neurological pathway or a single neuron. Even a small disturbance to a neuron's structural pathway can result in dysfunction. As a result, neurological disorders can result from a number of causes, such as, Lifestyle-related causes, infections, genetics, nutritional - related causes, environmental influences, physical injuries.

Signs of Neurological Disorders:

The signs of neurological disorders can vary significantly, depending upon the type of disorder as well as the specific area of the body that is affected. In some instances, you might experience emotional symptoms while in other cases physical symptoms may be the result.

Emotional symptoms: Depression or delusions

Physical symptoms: Partial or complete paralysis, Muscle weakness, Seizures, Difficult reading and writing, Partial or complete loss of sensation, Decreased alertness, Difficult reading and writing, Poor cognitive abilities.

Drug Addiction, Dependence and Withdrawal:[10]

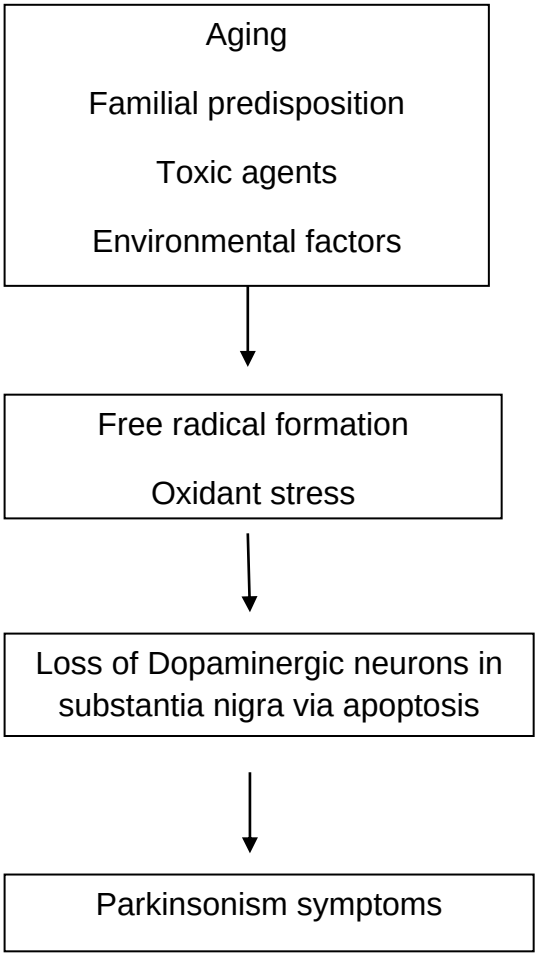
If a person developed a drug addiction, dependence and withdrawal are two important components. Dependence can develop when you take medication over a period of time. Depending on the addictive nature of the medication and your own personal situation, dependence can sometimes develop quickly. If the patient dependent on his medication, he will experience withdrawal symptoms when he abruptly stops taking the medication. Symptoms such as a headache, nausea and tremors occur.

1. Parkinsonism Disease:[11]

Parkinson's disease is a long-term degenerative disorder of the central nervous system that affects motor systems, often including tremors. Nerve cell damage in the brain (substantia nigra) causes dopamine levels to drop, leading to the symptoms of Parkinson's. Parkinson's often starts with a tremor in one hand. Other symptoms are slow movement (bradykinesia), difficult to walking, stiffness (Rigidity) and loss of

balance. Thinking and behavioural and include sensory, sleep, emotional problems may occur. It occurs more than 40 age people.

Pathophysiology of Parkinsonism Disease:[12]



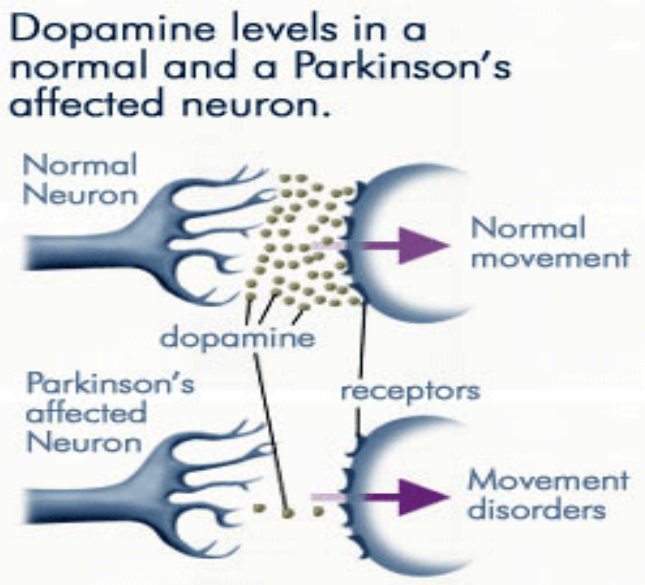


Fig No: 1. Dopamine concentration level

Symptoms: Parkinson's often starts with a tremor in one hand. Other symptoms are slow movement, stiffness, dizziness, amnesia, dementia, restless sleep, anxiety, impaired voice, reduced facial expression and depression.

More than 1 million cases per year having PD in India. levodopa, the most effective treatment for PD.

2. ALZHEIMER'S DISEASE:

Alzheimer's disease (AD) is a potential neurodegenerative disorder of nervous system which affects the primary areas of the brain dealing with learning and memory (**memory loss and cognitive decline.)** Deposition of the insoluble beta-amyloid protein on neurons activates the microglia cells which stimulate the process of neuroinflammation by releasing mediators. The release of cytokines and apoptotic promoters, in turn, shrink the neurons of the hippocampus leads to cognitive impairment. The disease starts mild and gets progressively worse.[13]

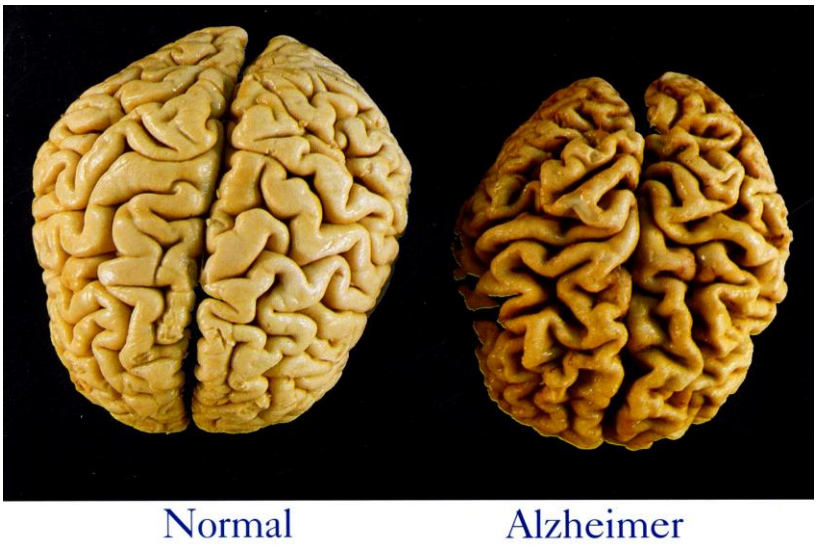
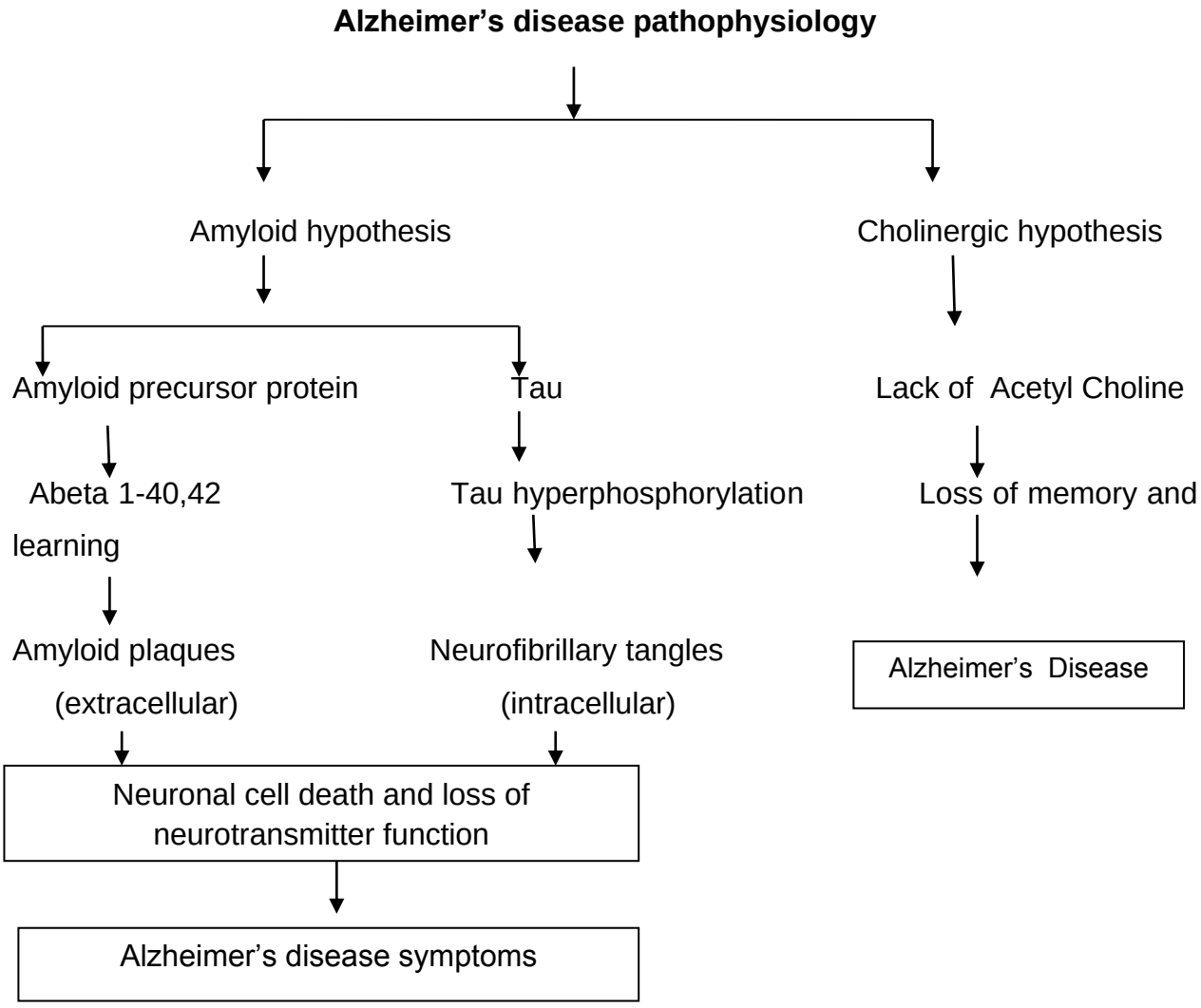


Fig No:2. Brain size shrinks in alzheimer's disease

Pathophysiology: Alzheimer's disease is the most common cause of dementia in people age 65 years and older. Dementia is a significant loss of cognitive functions such as memory, judgment, attention, and abstract thinking.

Acetylcholine (**ACh**) is a neurotransmitter essential for processing memory and learning, is decreased in both concentration and function in patients with **Alzheimer's disease**.(Irreversible dementia).

Brains of people with Alzheimer's disease contain clumps of a sticky substance known as amyloid and tangled strands of another protein formed when neurons die, called tau.



Symptoms: Patients suffering from AD can face serious behavioural problems which lead to the disorientation of time, space and place.

India has the world's lowest rate of Alzheimer's disease, Because of the assert extensive use of curcumin in India.It has a powerful antioxidant and possesses anti-inflammatory properties, which help fight the disease and symptoms. It mainly inhibits the accumulation of destructive beta amyloids-inert substance which is responsible for

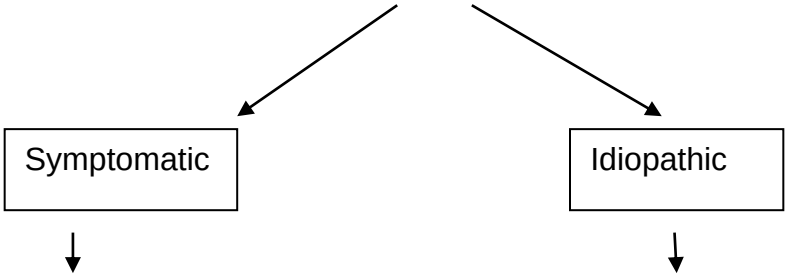
Alzheimer's disease.That's why India has the lowest rate of the disease in the world about 4.4 times less among adults aged 70-79 than the rate in us. In India, more than 4 million people have some form of dementia. Alzheimer's disease is usually caused by tangles and plagues in the brain, thus interrupting the normal cognitive functions of the brain and hence result in any of the above-mentioned symptoms. This disease begins to progress slowly in the brain which eventually makes the patient lose their entire memory. Usually, such disease takes place after a person crosses 60 years of age. There can occur strokes in the brain which are commonly known as vascular dementia.

In such cases, patients tend to remember nothing. They fail to remember even their names, recognize their relatives, their immediate family members such as brothers, sisters, parents etc. They begin to act just like a new born baby knowing nothing.

3. EPILEPSY: [14]

Epilepsy is a Chronic neurological disorder in which nerve cell activity in the brain is disturbed, causing seizures. Epilepsy may occur as a result of a genetic disorder or an acquired brain injury, such as a trauma or stroke. During a seizure, a person experiences abnormal behavior, abnormal motor activity (convulsion) and sensations, sometimes including loss of consciousness. The most common ages of incidence are under the age of 18 and over the age of 65.

Causes: The cause of an individual's epilepsy can be divided into two categories. [15]



1. Genetic conditions
2. Head injury
3. Bacterial or viral encephalitis
4. Complications during pregnancy or Birth.
5. Stroke

a disorder unto itself (cause unknown)

Stress Can Lead To Seizures:[16]

A new study finds that one-third of patients admitted to the epilepsy unit are not actually suffering from epileptic seizures: they're suffering from stress. This means that the seizures were essentially physical demonstrations of emotional stress, and not the result of abnormal electrical activity in the brain, as with epilepsy. The psychogenic seizures in these patients were strongly linked to life stresses.

Types of seizures:

1. Partial seizures: Partial seizures may be further subdivided into both simple and complex seizures. This refers to the effect of such a seizure on consciousness .Simple seizures cause no interruption to consciousness (although they may cause sensory distortions or other sensations), whereas complex seizures

disrupt consciousness to unreliable degrees. This does not inevitably mean that the person experiencing this sort of seizure will fall unconscious (like fainting).

2. Generalized seizures:

- Absence seizures involve an interruption to consciousness where the person experiencing the seizure seems to become vacant and unresponsive for a short period of time (usually up to 30 seconds). Slight muscle twitching may occur.
- Myoclonic seizure involve an extremely brief (< 0.1 seconds) muscle contraction and can result in jerky movements of muscles or muscle groups.
- Clonic seizures are myoclonus that is regularly repeating at a rate typically of 2-3 per second. in some cases, the length varies.
- Tonic-clonic seizure involve an initial contraction of the muscle (tonic phase) which may involve tongue biting, urinary incontinence and the absence of breathing. This is followed by rhythmic muscle contractions (clonic phase). This type of seizure is generally what is referred to when the term 'epileptic fit' is used colloquially.
- Atonic seizures involve the loss of muscle tone, causing the person to fall to the ground. These are sometimes called 'drop attacks' but should be well-known from similar looking attacks that may occur in cataplesy.

4. ANXIETY:

Anxiety disorders as such are a group of illnesses characterized by the presence of excessive worry, fear, tension, or activation that causes significant discomfort or a clinically significant deterioration of the activity of the individual. some of the physical symptoms, a fast heart rate, palpitation,tremor,sweating,dry mouth,chest pain, headaches, fast breathing, are partly caused by the brain which sends a lot of messages down nerves to different parts of the body when you are anxious. The nerve messages tend to make the heart, lungs, and other parts of the body work faster. In addition, you release stress hormones, such as adrenaline (epinephrine), into the bloodstream when you are anxious. These can also act on the heart, muscles and other

parts of the body to cause symptoms. About 1 in 20 people have an anxiety disorder at any one time. [17]

Anxiety is one of the most common mental disorders, characterized by changes in mood, behavior, somatic function, and cognition. Benzodiazepines and SSRIs are most commonly employed drugs for the treatment of anxiety. Synthetic drugs available for treatment of anxiety have various adverse effects. Drugs obtained from natural sources are known to cause fewer side effects compared to synthetic drugs despite same ability to cure disease.

Prevention, diagnosis and treatment of various illnesses are extensively practised by the traditional and alternative medicine. Over the past 20 years, this type of medicine has attracted public attention as it is easily accessible in some regions. As human diet consists of plant-derived foods, particularly vegetables and fruits are generally considered to be highly beneficial components, they contribute great importance in daily life by providing a wide range of nutrients, vitamins and other compounds which widen the therapeutic arsenal. Prevention of CNS related diseases by natural products plays a dominant role in the development of novel drug leads.

2.2 .REVIEW OF LITERATURE

Kavimani S, et al., (2014) reported the antioxidant and free radical scavenging activities of methanolic leaf and bark extracts of *DA* was evaluated by different in vitro antioxidant assay models. The total phenol and flavonoid content was also determined in the extracts. The plant extracts exhibited strong antioxidant and radical scavenging activity on ABTS radical cation, DPPH free radical, hydrogen peroxide, superoxide radical and hydroxyl radical. Both extracts showed strong activity in total reducing power assay. The antioxidant and free radical scavenging activities of the extracts were comparable to standard antioxidants used such as ascorbic acid and rutin. The extracts had good phenol and flavonoid contents. The antioxidant and radical scavenging activity of the plant extracts may be due to the rich amount of phenols and flavonoids. Therefore, the plant could be considered as a very good antioxidant source with therapeutic potential. [18]

Kavimani, et al.,(2014), carried out the In-Vitro anti-diabetic activity on *Dolichandrone atrovirens.* Diabetes mellitus is a metabolic disorder and one of the most critical complications of diabetes is postprandial hyperglycemia. Glucose metabolizing enzyme inhibitors are the class of compounds that help in managing postprandial hyperglycemia. This study reported that the methanolic leaf and bark extracts of *DA* for their efficacy to inhibit α-glucosidase, α-amylase and glucose-6-phosphatase in the in vitro systems. Potent and dose-dependent inhibition of these carbohydrate digestive enzymes was observed for both *DA* leaf and bark extracts and the observed results were comparable to standard drug acarbose and metformin. This enzyme inhibition provided a strong in vitro antidiabetic activity.[19]

Saminathan Kayarohanam, et al.,(2015), investigated the present study deals with the quantitative phytochemical and GC-MS analysis of aqueous methanolic bark and leaf extract of *DA*. This study concerns the quantitative screening of total glycosides, saponin,total phenol content, total flavonoid and vit c content. Further assessed the

DA leaf and bark extract using GC-MS. The preliminary screening test results in the detection of bioactive principles and GC-MS analysis revealed the presence of good compounds in leaf extract and 11 compounds in bark extract. This analytical technique identifies the presence of pharmacologically active constituents present in the metabolic aqueous bark and leaf extracts of *DA*. So that it can be recommended as a plant of phytopharmaceutical importance.[20]

Saminathan Kayarohanam , et al.,(2015), carried out the acute and sub-acute toxicity study of aqueous methanolic leaf and bark extract of *Dolichandrone atrovirens*. The acute toxicity studies of Aqueous methanolic *DA* leaf extract(DALE) and *DA* bark extracts(DABE) as a single dose(2000mg/kg) was administered to the swiss albino mice(20-25g) by oral route and the animals were observed for mortality and any toxic symptoms up to 14 days. In sub-acute toxicity studies, the DALE and DALE were administered daily for 28 days at doses ranging from 200-400 mg/kg. The animals were found in signs of toxicity, morbidity and mortality for 28 days. From the results, it was concluded that the dose of 400 mg/kg is safe for long-term treatment of diabetic conditions.[21]

P.Natarajan, et al., (2015), reported that the anticancer activity of the extract of *Dolichandrone atrovirens* (DA) has been evaluated against Dalton's ascitic Lymphoma (DAL) in swiss albino mice. It has a significant enhancement of mean survival time of treated groups tumor bearing mice was found with respect to DAL control group. Drug treatment was found to be an increase non-viable cell counts. After the treatment of extracts with DAL bearing mice shows that the tumour cell growth was found to be inhibited. After 14 days of inoculation, extracts is able to reverse the changes in the haematological parameters, liver function test and PCV consequent to tumour inoculation that near to the normal control group. The DA was reduced the ascitic fluid volume, viable count, and increased the percentage of lifespan of animals. This concluded DA having anticancer activity.[22]

Saminathan Kayarohanam, et al.,(2015), studied the phytochemical screening and HPLC analysis of bark and leaf extract of *Dolichandrone atrovirens*. This study involves the preliminary screening of carbohydrates, glycosides, protein, aminoacids, phenol, flavonoids, tannins, saponins, fixed oil and fats.*DA* leaf and bark extracts are further analyzed by HPLC using Shimadzu Class-VP V6.14 SP2system.The preliminary screening test results in the detection of bioactive principles and HPLC analysis conform the detection of bioactive principles and HPLC analysis conform the phytochemical active ingredients like gallic acid, rutin,quercetin,and ferulic acids are present in the leaf and extracts of *DA.*[23]

Saminathan Kayarohnam , et al.,(2015), reported that the histopathological studies of aqueous methanolic leaf and bark extract of *Dolichandrone atrovirens.* The present study was aimed to investigate the complications of untreated diabetes on histomorphology of rats. Diabetes mellitus was experimentally induced in male Wister rats by administration of streptozotocin. The establishment of diabetes mellitus was confirmed by fasting blood glucose levels. The animal was divided into five groups. Group one rat served as healthy controls that received the vehicle in a similar manner other four is treated by the aqueous methanolic leaf and bark extract *DA* given in the dose of 200 mg/kg, p.o and 400 mg/kg, p.o. For the histomorphological study of different organs, 50% of the animals were sacrificed after 14 days. The blood glucose level of diabetic rats was raised significantly throughout the experimental period. Further, histomorphological alterations were registered in kidneys and liver. The results show gross examination of liver and kidney did not show any abnormalities. It is clear from these results that *DA* leaves extract and bark is free from hepatic and renal toxicity.[24]

Asish tulshkar, et al.,(2011)studied the neuropharmacological activity of petroleum, chloroform and ethanolic extracts of aerial part of *lippia nodiflora linn,* With the experimental models using test such as potentiation of diazepam –induced sleeping time, locomotor activity, motor coordination, exploratory behavior

pattern,elevated plus maze and maximal electroshock convulsions. Diazepam at doses of 5,4, and 1 mg/kg served as standard.Results showed that the ethanolic extract of L.nodiflora at both doses(250 and 500 mg/kg p.o) and its chloroform extract at a higher dose of 500 mg/kg produced central inhibitory effects, anticonvulsant effect and anxiolytic effect in mice.[25]

Hitender Sharma, Munish Garg.,(2015), reported the neuropharmacological activities of *Taxus wallichiana* bark in Swiss albino mice. The sedative, motor coordination, anxiolytic, and antidepressant effects of the hydroalcoholic extract of *T. wallichiana* bark and its ethyl acetate fraction were evaluated in mice models of behaviour analysis. The effects were evaluated on diazepam-induced sleeping time elevated plus maze and light and dark box, and on the forced swimming test. General locomotor activity and motor coordination effects were evaluated in the actophotmeter and rota-rod tests respectively. Both the hydroalcoholic extract and ethyl acetate fraction showed a marked decrease in latency of sleep onset, prolonged the diazepam-induced sleeping time, decreased spontaneous locomotor activity; whereas ethyl acetate fraction produced anxiolytic and antidepressant activity.[26]

Mandar R Zambare , **et al,(2011),** reported the Study of central nervous system depressant and behavioural activity of an ethanol extract of *Achyranthes aspera* (Agadha) in different animal models. This study was to evaluated depressant effects on central nervous system (CNS) and behavioural effects of ethanol extract of A. *Aspera* (EEAA) and to find the phytochemical responsible for these activities. The pharmacological assays used to study CNS depressant effect in albino mice were rota rod and actophotometer performance test. Effects on behavioural activity were studied using open field test. The extract was given intraperitoneally (i.p.) at a dose of 400 mg/kg. Diazepam (2 mg/kg body weight i.p.) was used as a standard. The result of this study reflected that EEAA (400 mg/kg i.p.) decreased locomotor activity, produced muscle relaxation, and showed anxiolytic activity.[27]

Dilipkumar Pal, et al.,(2009), investigated the CNS depressant activity of roots of coccos nucifera in mice. The ethanolic extract of *coccos nucifera* (EECN) was tested for CNS depressant activity. EECN significantly potentiated the sleeping time of mice induced by standard hypnotics, diazepam. EECN showed significant analgesic properties as evidenced by the significant reduction in the number of writhes and stretches induced in mice by 1.2% acetic acid solution.It also potentiated analgesia induced by morphine and pethidine in mice. Pretreatment with EECN caused significant protection against pentylenetetrazole-induced convulsions.The behavioural studies on mice possessed CNS depressant activity of the ethanol extract of *cocos nucifera .*[28]

Hogade Maheswar,et al.,(2011), reported the Anticonvulsant activity of the whole plant of *Solanum surattense* was assessed by successive hot soxhlet extraction using petroleum –ether (40-60C), chloroform , and methanol respectively and finally with water maceration against MES and PTZ induced seizures in rats. The methanolic and Aqueous extracts showed significant ($p<0.01$) activity in MES-induced seizures by reducing tonic hind limb extension phase than pet-ether and chloroform when compare to control.Also methanolic and Aqueous extracts significantly ($P<0.01$) delayed the onset of clonic convulsions induced by Pentylenetetrazol. Thus Methanolic and Aqueous extracts of the whole plant of *Solanum surattense* possess the anticonvulsant activity.[29]

Ananda Kumar Shill , et al.,(2011), reported the Anti-inflammatory and neuropharmacological activities of *Caesalpinia pulcherrima* bark, The crude methanolic extracts of the bark of *Caesalpinia pulcherrima* were evaluated for its anti-inflammatory and neuropharmacological activities. When given orally to rats at a dose of 200 and 400 mg/kg, the extract showed a significant ($P < 0.001$) anti-inflammatory activity against carrageenin-induced paw oedema in rats comparable to the standard

drug phenylbutazone. The extract of *Caesalpinia pulcherrima* barks also potentiated the pentobarbital-induced sleeping time in mice and decreased the open field score in the open field test, decreased the number of hole crossed from one chamber in the hole cross test and decreased the head dip responses in hole board test.[30]

R.M. Perez G , et al.,(**1998)** reported that the neuropharmacological activity of *Solanum nigrum* fruit, The ethanol extract of the fruit of *Solanum nigrum* L. (Solanaceae) was studied for its neuropharmacological properties in experimental animals. On intraperitoneal injection, the extract significantly prolonged pentobarbital-induced sleeping time produced an alteration in the general behaviour pattern, reduced exploratory behaviour pattern, suppressed the aggressive behaviour, affected locomotor activity and reduced spontaneous motility. The observations suggest that the fruit of *S*. *nigrum* possesses potential CNS-depressant action. [31]

Mohammed saifuddin Khalid, et al.,(2013), reported the anti-anxiety effect of ethanolic extracts of *Agave americana* linn**,** the study of anti-anxiety effects of ethanolic extract of *Agave americana* (L) leaves in rat and mice. Diazepam 2mg/kg (p.o) was used as positive control. The animal treated orally (p.o) with a dose of 200 and 400 mg/kg of ethanolic extract. It showed significant action in the elevated plus maze, time spent and a number of entries in open, close arm. The hole board test showed a significant increase in the time spent in head dip latency, head dip count, rearing, 1st head dip latency, and decrease locomotion. In light dark model treatment with this extract showed an increase in time spent in light compartment, entries in light, dark compartment and number of the crossing. In social interaction, the ethanolic extract significantly increases social interaction time of low light, unfamiliar test condition. These results indicate an anxiolytic action from *Agave americana* (L) leaves extract on mice and rat, probably due to the action of flavonoid present in the *Agave americana* (L) leaves.[32]

Nahid Jivad, Zahra Rabiei,(2014), Studied a review on medicinal plants used in the treatment of learning and memory impairments Alzheimer's disease (AD) is a progressive brain disorder that gradually impairs memory and ability to learn, reasoning, judgment, communication and daily activities. AD'is characterized clinically by cognitive impairment and pathologically by the deposition of β amyloid plaques and neurofibrillary tangles and the degeneration of the cholinergic basal forebrain. During the progression of AD patients may produce changes in behaviour and personality.They studied the use of herbs in AD.[33]

Sunil k tomar , et al.,(2012), studied the skeletal muscle relaxant activity of chloroform extract of *phyllostachys bambusoides* on wistar rats.Skeletal muscle relaxant activity of the chloroform leaf extract of *Phyllostachys bambusoides* was investigated by testing the effects of the extract on wistar rat using rota-rod apparatus model, inclined screen test, climbing test. Experiments were carried out on male rat and the animals were randomly allotted to the different control and test groups. The extracts (chloroform) contain glycosides, carbohydrates, tannins, proteins and flavonoids. It was found that chloroform extracts up to a dose of 2000 mg/kg body weight, did not show any toxic manifestations or death. The extract was administered orally at a dose of 200 mg/kg. Diazepam in a dose of 4 mg/kg (s.c.) was used as a standard. Chloroform extract at the dose level of 200 mg/kg body weight showed significant skeletal muscle relaxant activity. On the bases of these results we can conclude that the *Phyllostachys bambusoides* may be used to develop herbal medicines against the same.[34]

Gummalla Pitchaiah, et al.,(2006) carried out the Anxiolytic and anticonvulsant activity of methanolic extract of Allium cepa Linn (Onion) bulbs in Swiss albino mice.the study was the anxiolytic and anticonvulsant activity of the methanolic extract of *Allium Cepa Linn* (MEAC). After preliminary phytochemical evaluation, acute oral toxicity test, anxiolytic activity of methanolic extract of *Allium Cepa* bulbs at doses of 200 and 400 mg/kg was assessed using elevated-plus-maze (EPM), open field test (OFT), light & dark transition (L&DT) models and anticonvulsant effect was assessed using maximal

electroshock (MES) and Isoniazid (INH) induced seizure models. Oral administration of MEAC for seven days significantly increased a number of entries and time spent in open arms in EPM model; latency, the number of squares crossed and time spent in Central Square in OFT; time spent in the light zone and the number of transitions in LDT model. Further, MEAC (200 and 400 mg/kg) showed a significant reduction in the duration of hind limb extensor phase in electroshock convulsions; protected the mice against the Isoniazid induced convulsions. Mechanistic studies showed significant improvement in brain GABA levels after *Allium cepa* treatment.[35]

Mohamed Nadjmouddine, et al.,(2015), Reported that the evaluation of anxiolytic activity of methanolic extract of *Urtica urens* in a mice model. The present study was designed to study the anxiolytic property of methanolic extracts of *Urtica urens;* an important and commonly used for its medicinal properties belongs to Urticaceae family. The anxiolytic activity was evaluated with the adult mice by hole board test, and the light–dark box test, and motor coordination with the rota rod test. The efficacy of the plant extract (100–400 mg/kg) was compared with the standard anxiolytic drug diazepam (1 mg/kg i.p.)The extract increased the time spent in the brightly-lit chamber of the light/dark box, as well as in the number of times the animal crossed from one compartment to the other. Performance on the rota rod was unaffected. In the hole board test, the extract significantly increased both head-dip counts and head-dip duration. *Urtica urens*, in contrast to diazepam, had no effect on locomotion. These results provides support for anxiolytic activity of *Urtica urens.*[36]

Dr. Deepa. Halemani, et al., (2011), carried out the Evaluation Of Anti-Anxiety Activity of Methanol Extract of *Aegle marmelos* (Bael Fruit Tree) leaves in Rats. The methanol extract of Aegle marmelos (AM, 70mg/kg, 140mg/kg and 210mg/kg), 2% of gum acacia, diazepam 2mg/kg are administered orally to randomly divided albino rats of either sex. Anxiolytic activity is assessed by Elevated plus maze (EPM) and Actophotometer (locomotor activity) models. Methanolic extract of Aegle marmelos showed significant anxiolytic activity at higher doses (210mg/kg).[37]

Ravindra C. sutar , et al.,(2014), carried out the evaluation of anticonvulsant activity of leaf extracts of H*oloptelea integrifolia* (roxb.) plant in experimental animals, The petroleum ether extract (100 and 300 mg/kg) and methanolic extract (300 mg/kg) delayed onset of PTZ- induced convulsions and also prolonged the onset of tonic convulsions in mice. Both the extracts failed to protect the rats from MES-induced convulsions. The extracts also protected rats against seizures induced by *lithium-pilocarpine*. In *Lithiumpilocarpine* model the petroleum ether extract (100 and 300 mg/kg) and methanolic extract (300 mg/kg) delayed the latency to rearing with forelimb clonus significantly.The results indicate that petroleum ether and methanol extracts contained such phytochemical compounds which are active in case of *Pentylenetetrazole* (PTZ) and *lithium-pilocarpine* induced status epilepticus, which support the ethnomedicinal application of the plant as an anticonvulsant agent.[38]

Nongnut Uabundit, et al.,(2010), investigated the Cognitive enhancement and neuroprotective effects of Bacopa monnieri in Alzheimer's disease model**.** The effect of alcoholic extract of *Bacopa monnieri* on cognitive function and neurodegeneration in an animal model of Alzheimer's disease induced by acetylcholine aziridinium ion (AF64A). Male Wistar rats were orally given the alcoholic extract of Bacopa monnieri at doses of 20, 40 and 80 mg/kg BW via feeding needle for a period of 2 weeks before and 1 week after the intracerebroventricular administration of AF64A bilaterally. Rats were tested for spatial memory using Morris water maze test and the density of neurons and cholinergic neurons was determined using histological techniques 7 days after AF64A administration. *Bacopa monnieri* extract improved the escape latency time ($p < .01$) in Morris water maze test.[39]

Nagaraja haleagrahara , et al.,(2009), studied the *Centella asiatica* extract (CAE)would prevent –methyl -4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- induced neurotoxicity in aged Sprague –Dawley rats.Adult , male Spraque-dawley rats of 300-350g were divided into control, *C.asiatic* alone,MPTP with C.asiatica (300mg/kg for 21 days)groups. Effect

of aqueous extract of C.asiatica on oxidative biomarker levels in corpus striatum and hippocampus homogenate was examined.MPTP challenged was elicited a significant increase in lipid hydroperoxides (LPO) ($p<0.01$),protein-carbonyl –content (PCC)($p<0.01$) and Xanthine oxidase(XO))($p<0.01$) when compared with control rats.There was a significant decrease in total antioxidants (TA)($P<0.001$),superoxide dismutase (SOD)($p<0.001$),glutathione peroxidase (GP_x)($p<0.01$) and catalase (CAT)($P<0.001$) levels with MPTP treatment .Supplementation of CAE reduced LPO and PCC and significantly increased ($P<0.01$)TA antioxidant enzyme levels ($P<0.01$)in corpus striatum and hippocampus.These results shows that administration of C.asiatica was effective in protecting the brain against neurodegenerative disorders such as parkinsonism.[40]

Stephane Bastianetto, et al.,(2006), investigated the neuroprotective effects of green and black teas and their catechin gallate esters against b-amyloid-induced toxicity. the deleterious role of b-amyloid (Ab) in the aetiology of Alzheimer's disease (AD), we investigated green and black tea extracts and flavan-3-ols (present as monomers and dimers in green and black forms, respectively) against toxicity induced by Ab-derived peptides using primary cultures of rat hippocampal cells as a model. Both green and black tea extracts displayed neuroprotective action against Ab toxicity. These effects were shared by gallic acid (1–20 lm), epicatechin gallate (ECG; 1– 20 lm) and epigallocatechin gallate (EGCG; 1–10 lm), the former being the most potent flavan-3-ol. In contrast, epicatechin and epigallocatechin were ineffective in the same range of concentrations. Moreover, only tea flavan-3-ol gallate esters (i.e. ECG, EGCG) and gallic acid inhibited apoptotic events induced by Ab25-35. Interestingly, EGCG and gallic acid inhibited Ab aggregation and ⁄ or the formation of Ab-derived diffusible neurotoxin ligands. Taken together, these results indicate that the catechin gallates (through the galloyl moiety) contribute to the neuroprotective effects of both green and black teas. Moreover, the protective effect of EGCG is likely to be associated, at least in part, with its inhibitory action on Ab fibrils⁄oligomers formation. These data also support the hypothesis that not only green but also black teas may reduce age-related neurodegenerative diseases, such as AD.[41]

Man-Shan Yu, et al.,(2005), studied the aged population dramatically increases in these decades, efforts should be made on the intervention for curing age-associated neurodegenerative diseases such as Alzheimer's disease (AD). Natural plant extracts of Lycium barbarum are well-known to exhibit anti aging effects. We, therefore, hypothesized that they exhibit neuroprotective effects against toxins in aging-related neurodegenerative diseases. In this study, we aimed to investigate whether extracts from L. barbarum have neuroprotective effects against toxicity of fibrillar Ab1–42 and Ab25–35 fragments. Primary rat cortical neurons exposed to Ab peptides resulted in apoptosis and necrosis. Pre-treatment with extract isolated from L. barbarum significantly reduced the release of lactate dehydrogenase (LDH). In addition, it attenuated Ab peptide-activated caspases- 3-like activity. The extract elicited a typical dose-dependent neuroprotective effect. The effective dosage of this extract was wider than that of a well-known western neuroprotective medicine lithium chloride (LiCl). We have further examined the underlying mechanisms of the neuroprotective effects. In agreement with other laboratories, Ab peptides induce a rapid activation of c-Jun N-terminal kinase (JNK) by phosphorylation. Pre-treatment of aqueous extract markedly reduced the phosphorylation of JNK-1 (Thr183/Tyr185) and its substrates c-Jun- I (Ser 73) and c-Jun-II (Ser 63). Taken together, we have proved our hypothesis by showing neuroprotective effects of the extract from L. barbarum. Study on anti-aging herbal medicine like L. barbarum may open a new therapeutic window for the prevention of AD.[42]

AIM

AND OBJECTIVES

2.2. AIM AND OBJECTIVES

Aim and objectives:

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Today, we are witnessing a great deal of public interest in the use of herbal remedies. Further more many western drugs had their origin in plant extract. There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. Given their potential to produce significant therapeutic effect, they can be useful as drug or supplement in the treatment / management of various diseases. Herbal drugs or medicinal plants, their extracts and their isolated compounds have demonstrated spectrum of biological activities.

Diseases of central nervous system are appearing as a major threat in the future because of increasing mental stress, strain and work, which are essential in the developing world. Nowadays neurological disorders are very common.(such as Psychosis, Alzheimer's disease, Parkinsonism disease, epilepsy, anxiety, etc..) People with these disorders are often subjected to social isolation, poor quality of life and increased mortality.

Herbal drugs are having diversified uses are always an alternative option to the synthetic drugs which are well known for their side and adverse effects. These reasons force the area of research to find improved treatments which will counteract the side effects and drawbacks of the existing treatment. Especially no one did the neuropharmacological activity in this plant.(DA). Based on the top of hypothesis the prospective medicinal plant specifically selected for investigation *DA* extracts for various experiments on swiss albino mice.

PLAN OF WORK

2.3. PLAN OF WORK

The Plan of present study is, the Neuropharmacological activity of *Dolichandrone atrovirens* in an animal model has been planned to be carried out in the following steps.

It is planned to carry out this work as outlined below.

1. Collection of literature review
2. Collection and authentication of the plant.
3. Extraction of leaves of DA plant using Soxhlet apparatus with using chloroform and ethanol as the solvent.
4. Phytochemical evaluation of chloroform and ethanolic extracts of *leaves of DA.*
5. Carrying out the TLC analysis for both plant extracts.
6. Evaluation of pharmacological activities of leaf extracts of the selected plant using various standard experimental models.
    i. To study the potentiation of diazepam-induced sleeping time:
    ii. To study the Spontaneous motor activity (SMA)-Actophotometer
    iii. To study the Motor coordination (Rota –rod)
    iv. To study the Exploratory behaviour pattern (Hole board test)
    v. To study the Maximal Electroshock-induced convulsion
    vi. To study the Light-Dark test

7. Histopathological studies

PLANT PROFILE

2.4. PLANT PROFILE

Fig No: 3. *Dolichandrone atrovirens*

PLANT PROFILE

SCIENTIFIC CLASSIFICATION:[43]

Name : *Dolichandrone atrovirens*

Kingdom : Plantae

Phylum : Tracheophyta

Class : Magnoliopsida

Order : Laminates

Family : Bignoniaceae

Genus : *Dolichandrone*

VERNACULAR NAMES :

Tamil : Poompadhiri (Pumpadiri),Perudi,Vattalappu

English : Indian trumpet flower, Wavy trumpet flower

Kannada : Paadri,beludura,belundare, Chithodi

Telugu : iruvoddi ,Oddi,Vadi,Vankaniroddi,

Botanical name : *Dolichandrone atrovirens*

PLANT DESCRIPTION:[44]

Dolichandrone atrovirens is commonly known as Wavy trumpet flower, belonging to the family Bignoniaceae. It is a deciduous tree, having 8-18 m height. The bark is rough thornless, and the branches are velvet hairy in nature.The leaves are

opposite,imparinnate,15-30cm long.The Flowers corolla white in colour, pedicles 1-3 cm long and the calyx is 2-2.5 cm long, lobes 5, rounded.

Useful parts:

leaf, bark, flower

Habit:

Tree

Tree type:[45]

Deciduous

Flower

Flowers are white in colour and trumpet in shape, flowering from April—May

Fruit

Fruit is a capsule and it is up to a foot long, brown, ribbed, seeds winged. Fruiting January—April

Leaf arrangement:

Opposite, imparipinnate,

Leaf type:

Hunse like

Leaf Apex:

Acuminate

Leaf Margin:

Entire

Habitat:

Dry deciduous forests

Global Distribution:

India

Indian Distribution:

Tamilnadu, Kerala, Andhra Pradesh, Maharastra, Karnataka

Propagation Techniques:

Direct sowing of seeds.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

3. Materials and Methods

3.1 Plant Material Collection and Authentication : The Leaves of the plant *Dolichandrone atrovirens* were collected locally from the campus of Kalasalingam University, Krishnankoil, srivilliputhur (Virudhunagar Dist, Tamilnadu) during the month of July 2016, It was then authenticated by Dr. D. Stephen, Professor, Department of Botany, The American College, Madurai.

Selection of animals: Inclusion criteria:

- Healthy swiss albino mice weighing 20-25 g of male mice with normal behaviour and activity1.
- Animals from JIPMER Institutional animal house (JIPMER, Puducherry-6, Tamilnadu.) were used for the study.

Exclusion criteria:

- Diseased animals are not included in the study.
- Animals used for other experiments within 4 weeks

Duration of study: 2 months Instruments required:

- Rota-rod
- Actophotometer
- Convulsiometer
- Hole board apparatus
- Light /dark compartment apparatus

Drug used for the studies:

Diazepam - Calmpose inj.(5mg/ml),Ranbaxy
Chloroform - CDH (Central Drug House, New Delhi)
Ethanol - CDH (Central Drug House, New Delhi)
Tween 80 - CDH (Central Drug House, New Delhi)
Silica gel (TLC grade) - CDH (Central Drug House, New Delhi)

3. 2 Preparation of Extraction:[46,47]

Extraction involves the separation of a bioactive portion of the plant tissues from the inactive components by using selective solvents in standard extraction procedure.

Method: About 800 gm of the dried powdered plant material was extracted successively with solvents of increasing polarity using soxhlet extractor.

Chloroform extract*:*

The dried coarse powder of leaves of *Dolichandrone atrovirens* was extracted with 1 litre of chloroform by using soxhlet apparatus, After 72 hours the extract was collected by filtration, the marc was separated for further extraction.The extraction was completed then it was allowed to distillation for the separation of solvent and make a acquire concentration. Finally, a dark green colour residue was obtained.

Ethanol extract*:*

The marc left after chloroform extraction, was dried and subsequently extracted with 1 litre of ethanol by using soxhlet apparatus. After the completion of extraction, it was filtered and the solvent was redistilled. Finally, a dark green colour residue was obtained.

Then a small fraction of all extracts was subjected to various chemical tests for the identification of various plant constituents as in the procedure given below and the findings are reported in table.2.

3.3 PRELIMINARY PHYTOCHEMICAL STUDIES

The chloroform and ethanol extracts of *Dolichandrone atrovirens* obtained were subjected to qualitative analysis to test the presence of various phytochemical constituents like alkaloids, carbohydrates, glycosides, flavonoids, steroids, aminoacid, phenols, proteins, tannins etc.

PROCEDURE:[48]

1. TESTS FOR CARBOHYDRATES:

A small portion of the chloroform, ethanol extract was dissolved separately in 5ml of water and filtered. The filtrate was subjected to the following tests.

a. **Molisch's test:** The extract mixed with a small amount of Molisch's reagent (α-naphthol dissolved in ethanol) in a test tube and add a small amount of concentrated sulfuric acid on the sides of the sloping test-tube to form a layer. The appearance of a purple ring at the user interface between the two layers indicates the carbohydrate presence.

b. **Fehling's test:** The extract taken in the test tube and add equal volumes of Fehling A (7% $Cuso_4$)&Fehling B(25%KOH and 35% sodium potassium tartrate) and place it in a boiling water bath for few minutes and see a change in colour.The brick-red precipitate indicates carbohydrate presence.

c. **Barfoed's test:** To the filtrate, Barfoed's solution(0.33molar solution of neutral copper acetate in 1% acetic acid) was added and it was boiled.The appearance of the Red colour precipitate.

d. Benedict's test:

To a small portion of the filtrate, Benedict's solution(anhydrous sodium carbonate, sodium citrate,copperII sulphate) was added and mixed well and it was boiled. Then it was allowed to cool.Red colour solution observed.

2. TEST FOR PROTEINS AND AMINO ACIDS

a. Ninhydrin Test:

Add two drops of 0.2% ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. The presence of proteins or amino acids produces a purple colour.

b. Biuret Test:

In the plant, extract add an equal amount of 5% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue colour is produced. Formation of pinkish or purple violet colour indicates the presence of proteins.

c. Millon's Test:

The extracts were added with millions reagent(mercuric nitrate in nitric acid) and it was boiled.The appearance of red colour shows the presence of proteins and free amino acids.

3. TESTS FOR ALKALOIDS: The small portion of the extracts were dissolved in suitable solvent and each extract was stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was tested for alkaloids by using the following reagents.

a. Mayer's test:

To 2-3 ml filtrate, add few drops Mayer's reagent (potassium mercuric iodide) formation of white precipitate indicates the presence of alkaloid.

b. Dragendroff's test:

To 2-3 ml filtrate, add few drops Dragendroff's reagent(potassium bismuth iodide) formation of orange-brown precipitate indicates the presence of alkaloids.

c. Wagner's test:

To 2-3 ml filtrate, add few drops Wagner's reagent(iodine+potassium iodide) formation of brown precipitate indicates the presence of alkaloids.

d. Hager's test:

To 2-3 ml filtrate, add few drops Hager's reagent (saturated picric acid) formation of yellow colour precipitate indicates the presence of alkaloids.

4. TEST FOR PHENOLIC COMPOUNDS AND TANNINS:

a. Braemer's test: To 3 ml of extract add dilute ferric chloride solution.The appearance of dark blue or greenish black colour indicates the presence of tannins.

b. To the extract add 10% Lead acetate solution. shake well. The appearance of white colour precipitate shows the presence of tannin.

5.TEST FOR TERPENOIDS

Add 0.5 gm of plant extract into 2 ml of chloroform and 3 ml concentrated sulphuric acid to form a layer. A reddish brown colour of the user interface indicates the presence of terpenoids.

6.TEST FOR FLAVONOIDS:

Shinoda test:

The extract of the plant taken in a test tube, add 5 ml of 95% ethanol with conc.HCl and magnesium turnings.The appearance of intense cherry red colour indicates the presence of flavonoids or orange-red colour indicates the presence of flavonols.

7. TEST FOR SAPONINS

a. Foam test/ Frothing test:

Add a small quantity of extract to 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

b. Haemolysis test:

The extracts of the plant were spread over a glass slide to form a thin film layer on which a drop of human blood was placed and spread over the extract layer. After 30 minutes, the slide was examined under a microscope for change in the structure and shape of red blood cells. Control was always maintained to see the change in red blood cells structure for haemolysis

8. TEST FOR FIXED OIL AND FATS

a. Spot Test: Extracts were taken and they were pressed between filter paper and the paper was noted.

b. Few drops of 0.5N alcoholic potassium hydroxide were added to various extracts with few drops of phenolphthalein. The mixture was heated on a water bath for 1-2 hours.formation of soap indicates the presence of fixed oils and fats.

9. TEST FOR GLYCOSIDES

a. Baljet' s Test: To 3ml extract added sodium picrate solution which gives orange colour shows the presence of glycosides.

b. Legal's Test: To 3ml extract, few ml of pyridine, 2 drops of nitroprusside and a drop of 20% NaOH solution were added which gives pink colour indicates the presence of glycosides.

c. Borntrager's Test: To 3ml extract was mixed with dilute.H_2So_4, and filtered. The filtrate was shaken with chloroform and the chloroform layer was separated. To this

dilute ammonia was added Which ammoniacal layer turns pink colour, indicates the presence of glycosides.

10. TEST FOR PHYTOSTEROLS:

The extracts were refluxed separately with a solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted with distilled water and extracted with ether. The etherial extract was evaporated and the residue was subjected to Libermann- Burchard test.

a. Libermann-Burchard test:

Extracts were shaken with few drops of dry acetic acid. To this, 3ml of acetic anhydride was added followed by 3 drops of conc. the sulphuric acid which gives green colour indicates the presence of phytosterol.

b. Salkowski reactions

To 2 ml of extract add 2 ml of chloroform and 2 ml of Con.H_2SO_4 which gives organic layer appear red colour shows the presence of phytosterol.

3.4. CHROMATOGRAPHIC TECHNIQUE - THIN LAYER CHROMATOGRAPHY

Separation and Isolation of plant constituents by chromatographic methods

The various methods of separating and isolating the plant constituents, the chromatographic procedure originated by Tswettis one of a most useful technique for general application. All finely divided solids have the powder to absorb other substances are capable of being adsorbed, some much more readily than others, this phenomenon of selective adsorption is the fundamental principle of chromatography. In the present study, thin layer chromatography methods were used.

3.4.1. Thin Layer Chromatography (TLC)

Thin Layer Chromatography is so widely used that it has become an essential technique for analyst and research workers.TLC is the almost universal analytical technique in chemical analysis for the organic and inorganic matter.TLC is a simple and rapid method carried out using a thin layer of adsorbents on plates.TLC not only combines the advantage of paper and column chromatography but in certain aspects it is found to be the superior method.

TLC is an important tool in the separation, identification and estimation of different classes of natural products. When a mixture containing different components is made to ascend in a TLC plate with the help of a solvent which acts as mobile phase, there will be a preferential adsorption of different components at different places on the plate.The result is the separation of components.

Preparation of TLC Plate:

80 gm of silica gel G was weighed and shaken to a homogenous suspension with 85 ml of distilled water for 90 seconds. This suspension was poured in TLC applicator which was adjusted to 0.25 mm thickness 20 carriers the transparence of layer disappeared. The plates were dried in hot air oven at 110^0 C for 30 minutes

(activation).The plates were then stored in a dry atmosphere and used whenever required.

Application of extracts for separation:

The various diluted extracts spotted on a TLC plate 2 cm above its bottom using the capillary tube. The most solution for application was between 0.1-1% strength. The starting point was equally sized as far possible and spots had a diameter ranging from 2-5 mm.

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

The various extracts of leaves of *Dolichandrone atrovirens* were subjected to thin layer chromatography using different mobiles phases that are suitable for detecting various phytoconstituents like alkaloids, glycosides, flavonoids, tannins and phenols of the two extracts, chromatogram for the chloroform,ethanol was carried out using the procedure recommended by Indian pharmacopoeia.

3.5 ACUTE TOXICITY STUDIES

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time (usually less than 24 hours).It is called as acute toxicity. The adverse effects should occur within 14 days of the administration of the substance.[49]

Acute Toxicity Study

Acute oral toxicity test was carried out as per the Organization for Economic Co-operation and Development (OECD-423) guidelines for testing of chemicals. Six randomly selected male mice were used for the acute toxicity study of each extract.[50] For a sighting study, a single mice was fasted overnight and administered with a dose of 2000 mg/kg of each extract orally by gavage. Then, the mice also fasted for 4 h with no access to food after extract administration. Immediately after dosing, the animals were observed continuously for the first 4h with 30 min intervals and until 24 h for any behavioral changes (paw-licking, motor activity, tremors, convulsions, posture, spasticity, ataxia, sensations, ptosis, lacrimation, exophthalmos, salivation, diarrhoea, writhing, skin colour, respiratory rate and mortality) and sign of toxicity.[51] Since no death was observed within 24 h, additional two animals were added to each extract and administered the same dose. The animals were observed continuously for 4 h with 30 min interval and then for 14 consecutive days with an interval of 24 h.

None of the group did not produce any apparent gross effect on general motor activity, faecal output, muscular weakness, tremors, convulsions, death ,etc .during the period of observation. This indicated that the extracts were considered to be safe at the tested dose level.

CHAPTER IV

PHARMACOLOGICAL EVALUATION

CHAPTER IV

4. PHARMACOLOGICAL EVALUATION

4.1.Neuropharmacological activity:

1. Potentiation of diazepam-induced sleeping time:[52]

This test is based on to evaluate the CNS properties of drugs in animal models. Many of the pharmacological tests are based on the potentiation of sleeping time by induced by barbiturates or other sedative drugs. The sleep evaluation method was based on the prolongation of diazepam-induced sleeping time.

The animals were divided into six groups ,each group containing five mice. The groups are as follows:

Group I : Control group of animals received vehicle (2.5% tween 80)

Group II : Treated with diazepam (2 mg/kg) i.p

Group III : Treated with chloroform leaves extract of *Dolichandrone atrovirens*(CEDA) (200 mg/kg) p.o and diazepam (2 mg/kg) i.p

Group IV : Treated with Chloroform extract of leaves of *Dolichandrone atrovirens*(CEDA)(400 mg/kg) p.o and diazepam (2 mg/kg) i.p

Group V : Treated with ethanolic extract of leaves of *Dolichandrone atrovirens*(EEDA)(200 mg/kg) p.o and diazepam (2 mg/kg) i.p

Group VI : Treated with ethanolic extract of leaves of *Dolichandrone atrovirens*(EEDA)(400 mg/kg) p.o and diazepam (2 mg/kg) i.p

The two parameters were observed after administration of test drugs. Time elapsed since the application of diazepam until the loss of the righting reflex (latency /onset of action) and the time elapsed from the loss to regaining of the righting reflex (duration of sleep). Early onset and/or prolonged sleep compared to control group that indicates the potential of sedative , hypnotic activity in the test drug.

2.Spontaneous motor activity (SMA)

The spontaneous locomotor activity was monitored by using Digital Actophotometer, which is based on the principle that when there is a breaking of infrared beams, the instrument registers locomotion of experimental animals automatically. In this method CNS depressant or stimulant, property was evaluated.

The animals were divided into six groups each group containing five mice. The groups are as follows:

Group I : Control group of animals received vehicle(2.5 % tween 80)

Group II : Treated with diazepam (2 mg/kg) i.p

Group III : Treated with Chloroform leaves extract of *Dolichandrone atrovirens*(CEDA)(200 mg/kg) p.o

Group IV : Treated with Chloroform extract of leaves of *Dolichandrone atrovirens*(CEDA)(400 mg/kg) p.o

Group V : Treated with Ethanolic extract of leaves of *Dolichandrone atrovirens*(EEDA)(200 mg/kg) p.o

Group VI : Treated with Ethanolic extract of leaves of *Dolichandrone atrovirens*(EEDA)(400 mg/kg) p.o

The locomotor activity for each animal was automatically recorded and after the treatment at 30 min intervals for a total of 120 min. Results of the treated groups were compared with those of the control group at each time interval.

3. Motor coordination [53]

Motor coordination test for the mice was evaluated by using Rotarod apparatus. This apparatus used to study the activity of drug interfering with motor coordination. Mice were placed on a horizontal steel rod rotating at the speed of 16 rpm for a period of 5 min. The mice capable of remaining on the top for 3 min or more, in three successive trials were selected for the study.

The animals were divided into six groups each group containing five mice. The groups are as follows:

Group I : Control group of animals received vehicle(2.5 % tween 80)

Group II : Treated with diazepam (2 mg/kg) i.p

Group III : Treated with Chloroform leaves extract of *Dolichandrone atrovirens*(CEDA)(200 mg/kg) p.o

Group IV : Treated with Chloroform extract of leaves of *Dolichandrone atrovirens*(CEDA)(400 mg/kg) p.o

Group V : Treated with Ethanolic extract of leaves of *Dolichandrone atrovirens*(EEDA)(200 mg/kg) p.o

Group VI : Treated with Ethanolic extract of leaves of *Dolichandrone atrovirens*(EEDA)(400 mg/kg) p.o

Inability to remain on the rod at least for 3 min was considered as a positive test and the fall of time of mouse was recorded at a time interval of 30 min up to 120 mins.

4.Exploratory behavior pattern (Hole board test)

The **hole-board test** (HBT) is an experimental method used in scientific research to measure anxiety, stress, and emotionality in animals. it is a popular test in behavioral pharmacology. The hole board apparatus consists of wooden box with 16 equidistant holes 3 cm in diameter in the floor. The apparatus was elevated to the height of 25cm.

The animals were divided into six groups each group containing five mice.The groups are as follows:

Group I	:	Control group of animals received vehicle(2.5 % tween 80)
Group II	:	Treated with diazepam (2 mg/kg) i.p
Group III	:	Treated with Chloroform leaves extract of *Dolichandrone atrovirens*(CEDA)(200 mg/kg) p.o
Group IV	:	Treated with Chloroform extract of leaves of *Dolichandrone atrovirens*(CEDA)(400 mg/kg) p.o
Group V	:	Treated with Ethanolic extract of leaves of *Dolichandrone atrovirens*(EEDA)(200 mg/kg) p.o
Group VI	:	Treated with Ethanolic extract of leaves of *Dolichandrone atrovirens*(EEDA)(400 mg/kg) p.o

The numbers of head pokes (head dipping) during a period of 5 min was recorded. The head dipping was recorded when both the eyes disappeared into the hole.

5. Maximal Electroshock induced convulsion

The electroshock induced convulsion method was effective in grandmal epilepsy. . The tonic convulsions of the hind extremities of the mice were induced by passing the current 150mA for 0.2 seconds duration through electro convulsiometer using corneal electrodes, after 60 min of oral administration of plant extract or vehicle or diazepam.

The animals were divided into six groups, each group containing five mice. The groups are as follows:

Group I	:	Control group of animals received vehicle(2.5 % tween 80)
Group II	:	Treated with diazepam (2 mg/kg) i.p
Group III	:	Treated with Chloroform leaves extract of *Dolichandrone atrovirens*(CEDA)(200 mg/kg) p.o
Group IV	:	Treated with Chloroform extract of leaves of *Dolichandrone atrovirens*(CEDA)(400 mg/kg) p.o
Group V	:	Treated with Ethanolic extract of leaves of *Dolichandrone atrovirens*(EEDA)(200 mg/kg) p.o
Group VI	:	Treated with Ethanolic extract of leaves of *Dolichandrone atrovirens* (EEDA)(400 mg/kg) p.o

The incidence and duration of extensor tonic was noted. A complete abolition of hind limb tonic extension was considered as 100 % protection of drug.

6. Light-Dark test[54]

The light/dark transition test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodent in response to mild stressor. The apparatus used for the light/dark transition test consisted of a cage divided into two sections of equal size. One of which light while the was dark.

The animals were divided into six groups each group containing five mice. The groups are as follows:

Group I : Control group of animals received vehicle(2.5 % tween 80)

Group II : Treated with diazepam (2 mg/kg) i.p

Group III : Treated with Chloroform leaves extract of *Dolichandrone atrovirens*(CEDA)(200 mg/kg) p.o

Group IV : Treated with Chloroform extract of leaves of *Dolichandrone atrovirens*(CEDA)(400 mg/kg) p.o

Group V : Treated with Ethanolic extract of leaves of *Dolichandrone atrovirens*(EEDA)(200 mg/kg) p.o

Group VI : Treated with Ethanolic extract of leaves of *Dolichandrone atrovirens*(EEDA)(400 mg/kg) p.o

Each animal was placed individually at the centre of the light-dark compartment, recordings were made over a 5 minute period. The following parameter were recorded,

1. The number of transition between the light and the dark compartment (tunnel crossing)
2. The total time spent in the light compartment.

4.2. HISTOPATHOLOGICAL STUDIES

Histopathology study of brain: [55,56]

The central nervous system consists of neurons and supporting cells called glia. In the brain, specialized regions such as the area postrema, ventricular lining and associated structures such as leptomeninges each have their particular morphology and reaction patterns in the event of injury.

A brain of each group mouse was fixed in 10% formalin and preceded for histopathological studies for evaluation of brain neuronal damage of all the group animals.

CHAPTER V

RESULTS

5.EXPERIMENTAL RESULTS

5.1.The yield of Extraction of the *Dolichandrone atrovirens* leaves

Table No:1.The yield of Extraction of the *Dolichandrone atrovirens* leaves

S.No	Extracts	Colour and Consistency	Percentage yield of Extracts of *Dolichandrone atrovirens* w/w
1	Chloroform	Dark green with viscous mass	1.85
2	Ethanol	Dark green with sticky mass	0.95

5.2.Preliminary phytochemical screening

Table No:2. Phytochemical screening of chloroform, ethanolic leaf extracts of *Dolichandrone atrovirens*

Phytochemical constituents	Chloroform extract	Ethanolic extract
Carbohydrates	+	+
Glycosides	+	+
Proteins & Aminoacids	+	+
Tannins & phenolics	+	+
Terpenoids	+	+
Flavonoids	+	+
Phytosterols	-	+
Fixed oils &Fats	+	+
Alkaloids	-	+
Saponins	+	+
Gums & Mucilages	-	-

(+) = indicates the presence of constituents, (-) = indicates the absence of constituents

5. 3. CHROMATOGRAPHY –THIN LAYER CHROMATOGRAPHY

5.3.1. TLC of Chloroform extract of *Dolichandrone atrovirens*

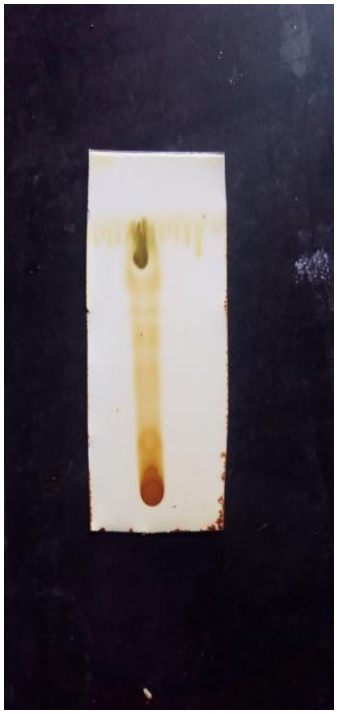


Fig No: 4. TLC of chloroform extract of *DA*

$$R_f \text{ Value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

$$= 4.8 / 6$$

$$= 0.8$$

5.3.2.TLC of Ethanolic extract of *Dolichandrone atrovirens*

Fig No: 5. TLC of ethanolic extract of *DA*

$$R_f \text{ Value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

$$= 5.4 / 6.1$$

$$= 0.88$$

5.3.3. R_f Value of extracts *of Dolichandrone atrovirens* leaves

Table No:3. R_f Value of extracts *of Dolichandrone atrovirens* leaves

S.No	Extracts	R_f Value
1	CEDA	0.8
2	EEDA	0.88

5.4.PHARMACOLOGICAL EVALUATION RESULTS

Table No:4

5.4.1. Potentiation of diazepam –induced sleeping time:

Group	Treatment	Onset of action (mins)	Duration of action (mins)
I	Control	7.5 ± 1.15	53.6 ± 5.6
II	Diazepam(2 mg/kg)i.p	3.08 ± 0.79**	101.8 ± 4.81**
III	Chloroform extract (200 mg/kg) p.o+diazepam(2 mg/kg) i.p	6.3 ± 0.82	51.4 ± 2.1
IV	Chloroform extract (400 mg/kg) p.o+diazepam(2 mg/kg) i.p	5.27 ± 0.17*	70.2 ± 3.27
V	Ethanolic extract (200 mg/kg) p.o+diazepam(2 mg/kg) i.p	5.14 ± 0.38*	73.2 ± 5.06*
VI	Ethanolic extract (400 mg/kg) p.o+diazepam(2 mg/kg) i.p	4.45 ± 0.36**	90.2 ± 5.54**

Results are expressed as mean ± SEM,(n=5), from six observations as compared to standard group the one way ANOVA followed by Dunnett's test. **Values are significantly different at P<0.001, * Values are significantly different at P<0.01

Effects of chloroform and ethanolic extracts of *DA* leaves on diazepam induced sleeping time

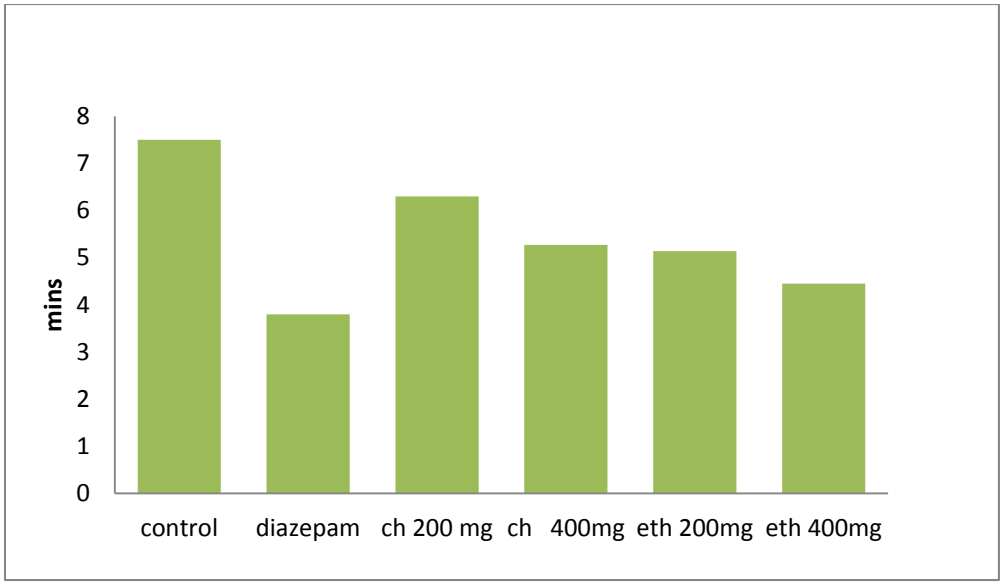


Fig No:6. Onset of action of mice in potentiation of diazepam inducing sleeping time

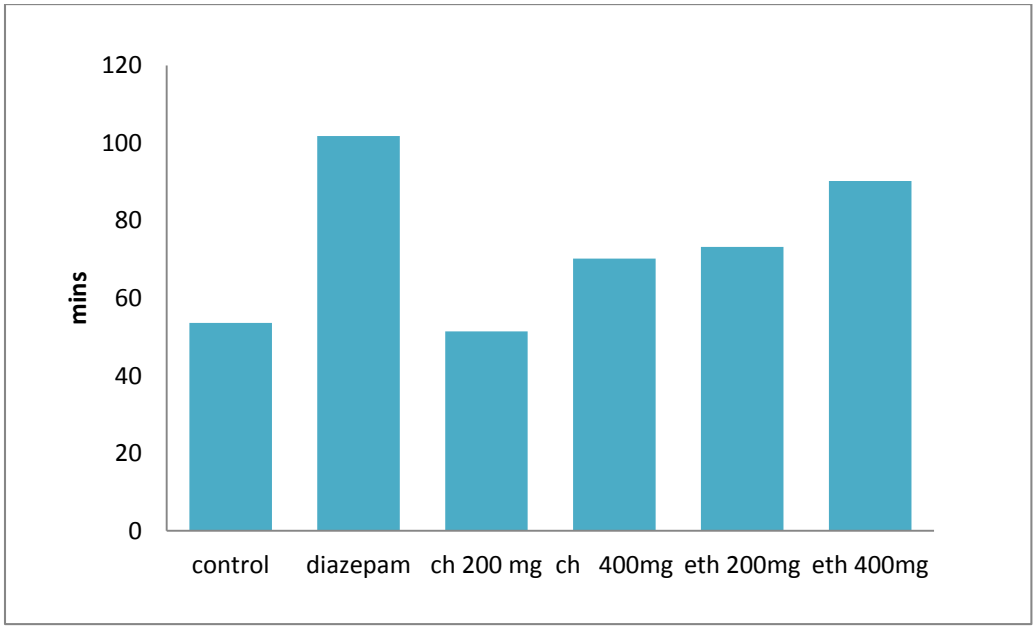


Fig No:7. Duration of action of mice in potentiation of sleep inducing diazepam

5.4.2. Spontaneous motor activity (SMA):

Table No:5

Group	Treatment	Experimental mean time(5 min)				
		0	30	60	90	120
I	Control	315.25±8.63	314.05±5.22	305.95±6.73	311.75±8.33	305.7±14.09
II	Vehicle and diazepam(2 mg/kg) i.p	312.6±9.34	118.4±12.91**	59.15±7.35**	42.05±8.05**	64.4±15.24*
III	Chloroform extract (200mg/kg) p.o	319.4±3.64	221.8±3.70	145.50±13.0*	131.65±7.19	162.3±5.26
IV	Chloroform extract (400mg/kg) p.o	322.8±6.49	183.4±8.08*	138.6±14.79	123.6±13.09	145.45±10.26
V	Ethanolic extract (200mg/kg) p.o	321.1±4.56	176.4±10.94*	126±28.01**	89.1±7.07	120.4±8.93
VI	Ethanolic extract (400mg/kg) p.o	315.4±11.56	148.65±12.2**	111.35±6.84**	55.9±9.48**	108.1±11.31

Results are expressed as mean ± SEM,(n=5), from six observations as compared to standard group the one way ANOVA followed by Dunnett's test. ****** Values are significantly different at P<0.001**, *** Values are significantly different at P<0.01

Effects of chloroform and ethanolic extracts of *DA* leaves on spontaneous motor activity using digital Actophotometer

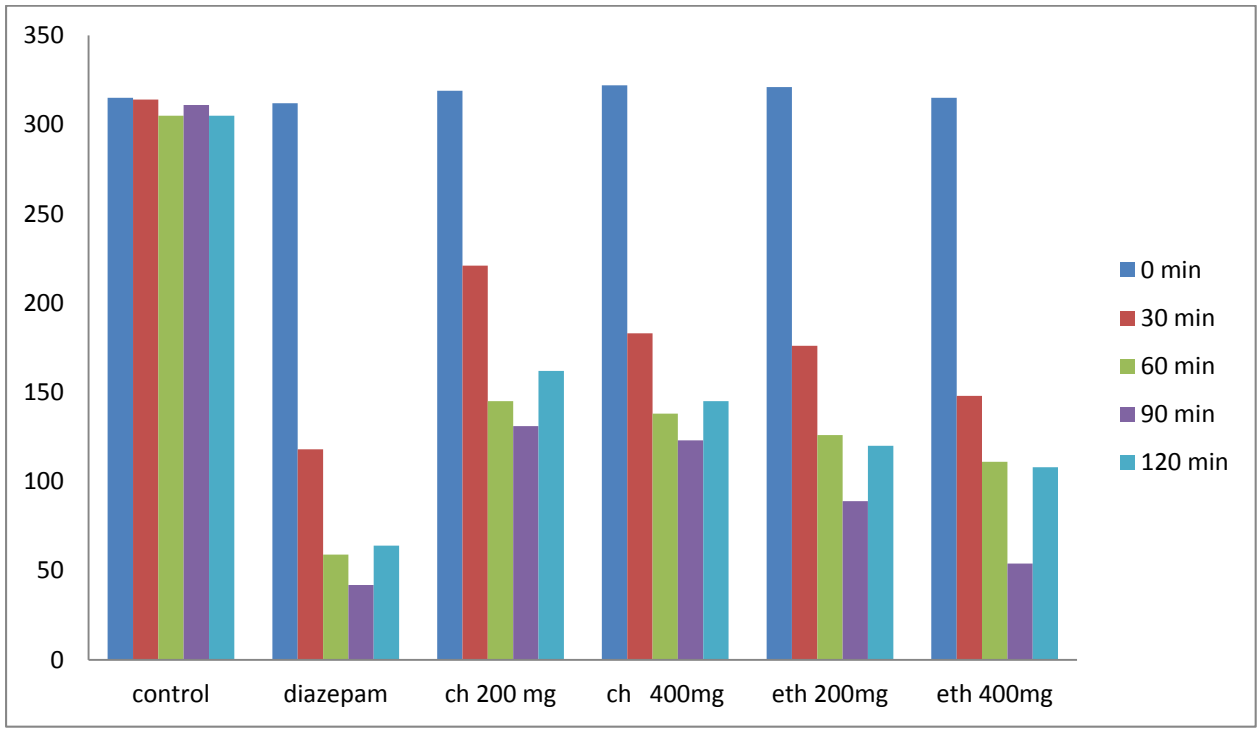


Fig No: 8. Experimental mean time (3 min) in spontaneous motor activity in digital Actophotometer

5.4.3.Motor coordination

Table No:6

Group	Treatment	Time spent on rods(5min)				
		0	**30**	**60**	**90**	**120**
I	Control	212.7±9.91	214.6±5.60	212.6±2.54	216.3±5.41	217±11.84
II	Vehicle and diazepam(2 mg/kg)i.p	212.5±5.89	48.95±11.31**	25.75±6.41**	74±7.24**	106.4±7.40**
III	Chloroform extract (200mg/kg) p.o	216.1±3.47	132±9.10	91.6±14.1	125.8±6.49	162.4±7.43
IV	Chloroform extract (400mg/kg) p.o	215.85±4.92	120±15.85*	74±12.38*	113.3±9.73	150.6±5.94
V	Ethanolic extract (200mg/kg) p.o	213.25±8.36	85±21.38	63.8±8.46**	101.95±9.5	123.4±6.06
VI	Ethanolic extract (400mg/kg) p.o	208.2±4.95	77.4±51.28	45±7.84**	88.1±4.97**	114±7.34

Results are expressed as mean ± SEM,(n=5), from six observations as compared to standard group the one way ANOVA followed by Dunnett's test. ****** Values are significantly different at P<0.001 **, *** Values are significantly different at P<0.01

Effects of chloroform and ethanolic extracts of *DA* leaves on Rota-rod apparatus for motor co-ordination

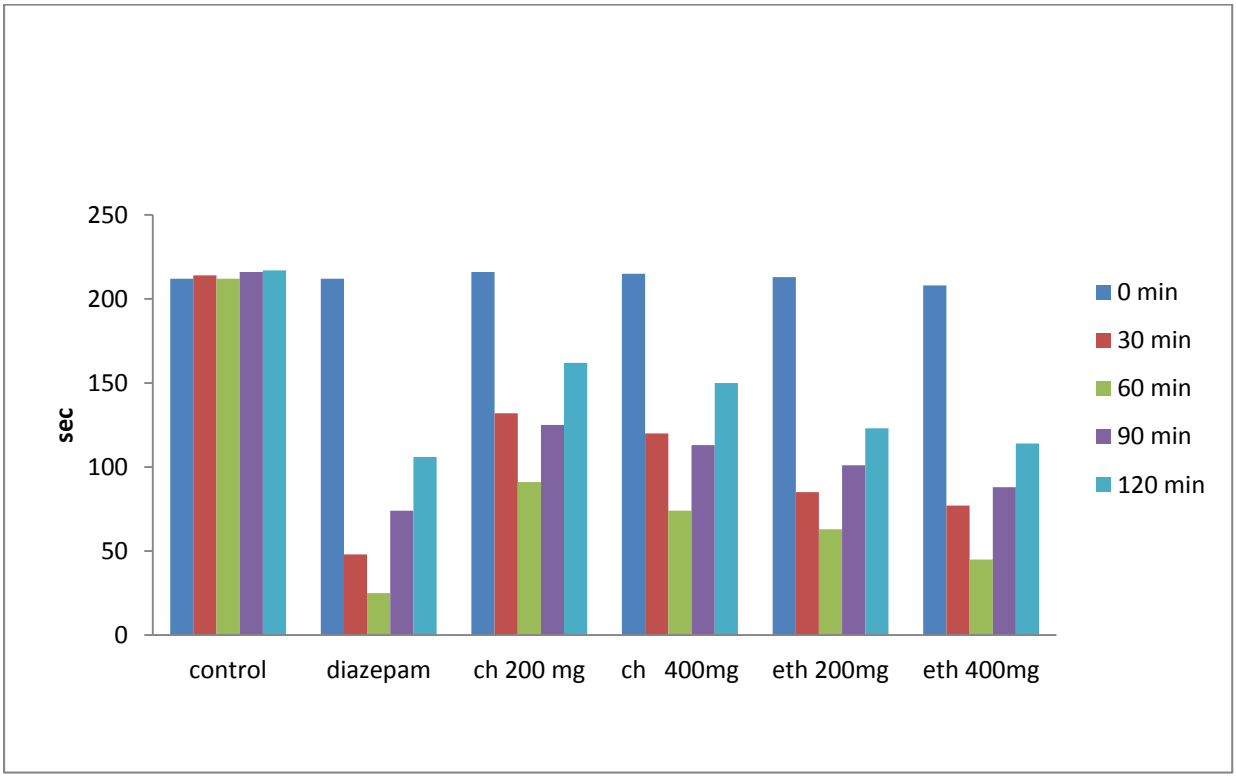


Fig No:9. Time spent mice on rods in Rota rod apparatus

5.4.4.Exploratory behavior pattern (Hole board test)

Table No:7

Group	Treatment	Number of head dipping in 5 mins	
		Before treatment	**After treatment**
I	Control	18±2.34	18±0.89
II	Vehicle and diazepam(2mg/kg)i.p	18±1.48	24±1.51**
III	Chloroform extract (200mg/kg)p.o	16±1.67	23±1.14
IV	Chloroform extract (400mg/kg)p.o	18±1.58	25±2.23*
V	Ethanolic extract (200 mg/kg)p.o	17±1.73	29±1.14**
VI	Ethanolic extract (400mg/kg)p.o	19±1.92	33±2.04**

Results are expressed as mean ± SEM,(n=5), from six observations as compared to standard group the one way ANOVA followed by Dunnett's test. ****** Values are significantly different at P<0.001 , ***** Values are significantly different at P<0.01

Effects of chloroform and ethanolic extracts of *DA* leaves on Hole board test

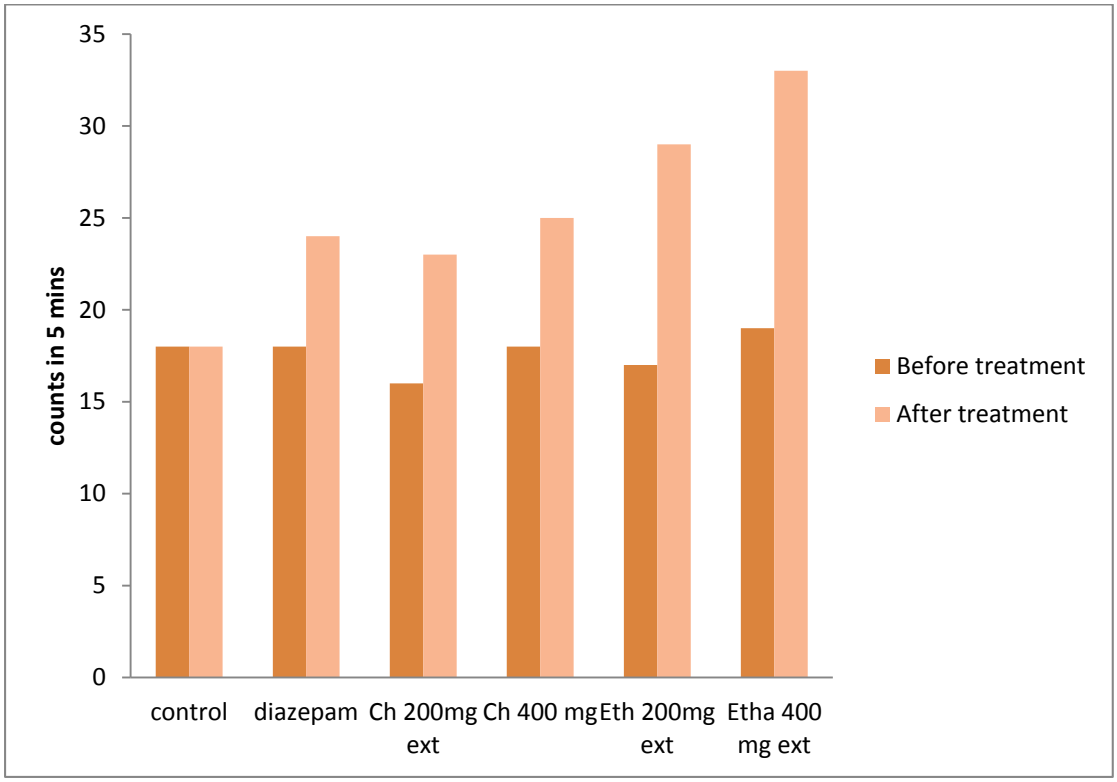


Fig No:10. Number of head dipping counts in 5 mins

5.4.5.Maximal Electroshock induced convulsion

Table No:8

Group	Treatment	Duration of tonic hind limb extension(sec)	Incidence of convulsions
I	Control	28.3± 4.89	8/8
II	Vehicle and diazepam(2mg/kg) i.p	5± 0.71**	2/8
III	Chloroform extract (200mg/kg)p.o	27± 1.24	7/8
IV	Chloroform extract (400mg/kg)p.o	25.5± 1.27*	6/8
V	Ethanolic extract (200mg/kg) p.o	18± 1.58*	5/8
VI	Ethanolic extract (400mg/kg) p.o	13.5± 0.95**	3/8

Results are expressed as mean ± SEM,(n=5),****** from six observations as compared to standard group the one way ANOVA followed by Dunnett's test. Values are significantly different at P<0. ***** Values are significantly different at P<0.01.

Effects of chloroform and ethanolic extracts of *DA* leaves on convulsiometer

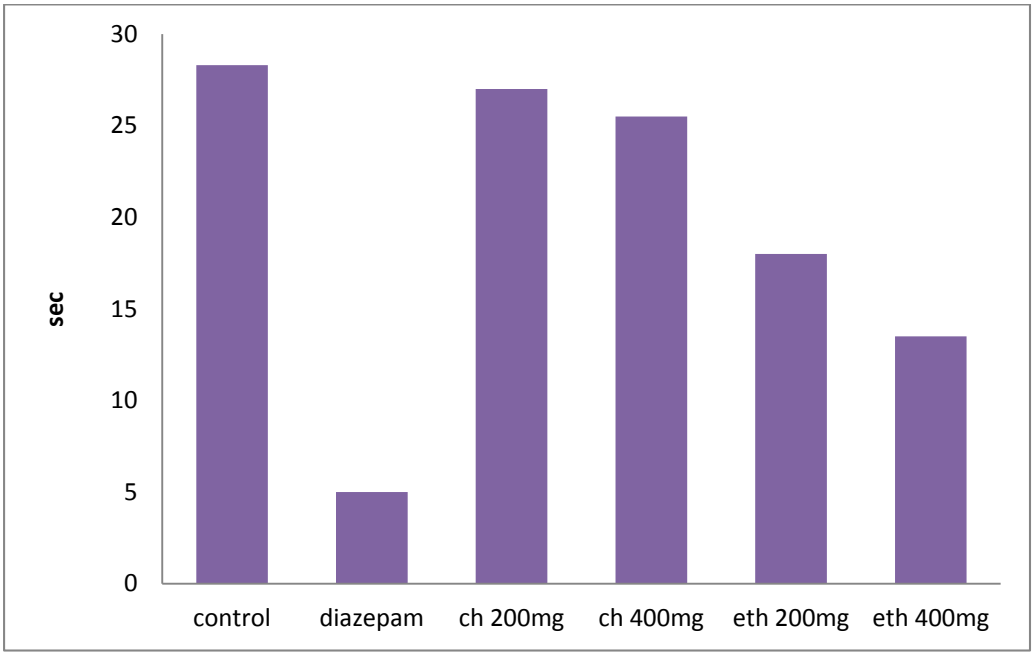


Fig No:11. Duration of tonic hind limb extension

5.4.6.Light-Dark test

Table No:9

Group	Treatment	Time spent in light area (sec)	Number of transition between light and dark(or)tunnel crossing
I	Control	97.2± 11.82	15± 2.730
II	Vehicle and diazepam(2mg/kg)i.p	186.2± 9.17**	15.4± 2.30**
III	Chloroform extract (200 mg/kg)p.o	129.8± 7.85	25± 1.58
IV	Chloroform extract (400 mg/kg)p.o	141.6± 8.44	22± 1.58
V	Ethanolic extract (200 mg/kg)p.o	157± 7*	18± 0.83**
VI	Ethanolic extract (400 mg/kg)p.o	163.4± 8.20**	17.6± 2.07**

Results are expressed as mean ± SEM,(n=5), from six observations as compared to standard group the one way ANOVA followed by Dunnett's test. ****** Values are significantly different at P<0.001, ***** Values are significantly different at P<0.01

Effects of chloroform and ethanolic extracts of *DA* leaves on light-dark test

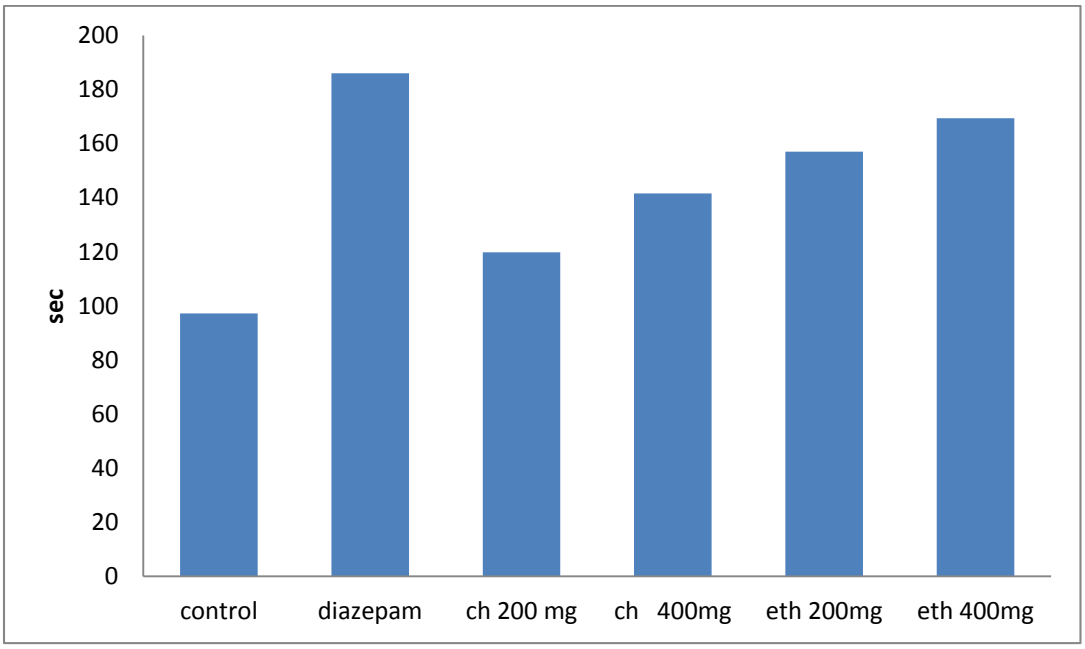


Fig No:12. Time spent in light area in light/dark test

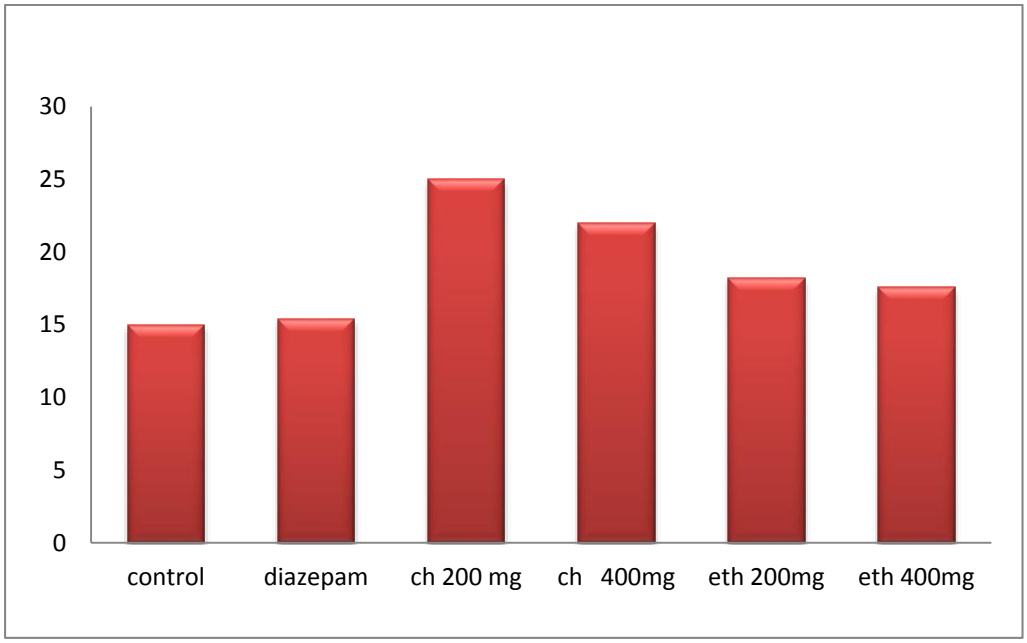


Fig No:13.Number of transition between light/dark compartment

HISTOPATHOLOGICAL REPORT

The histopathological evaluation of brain neuronal in all groups was examined and shown in figures. The description is as follows,

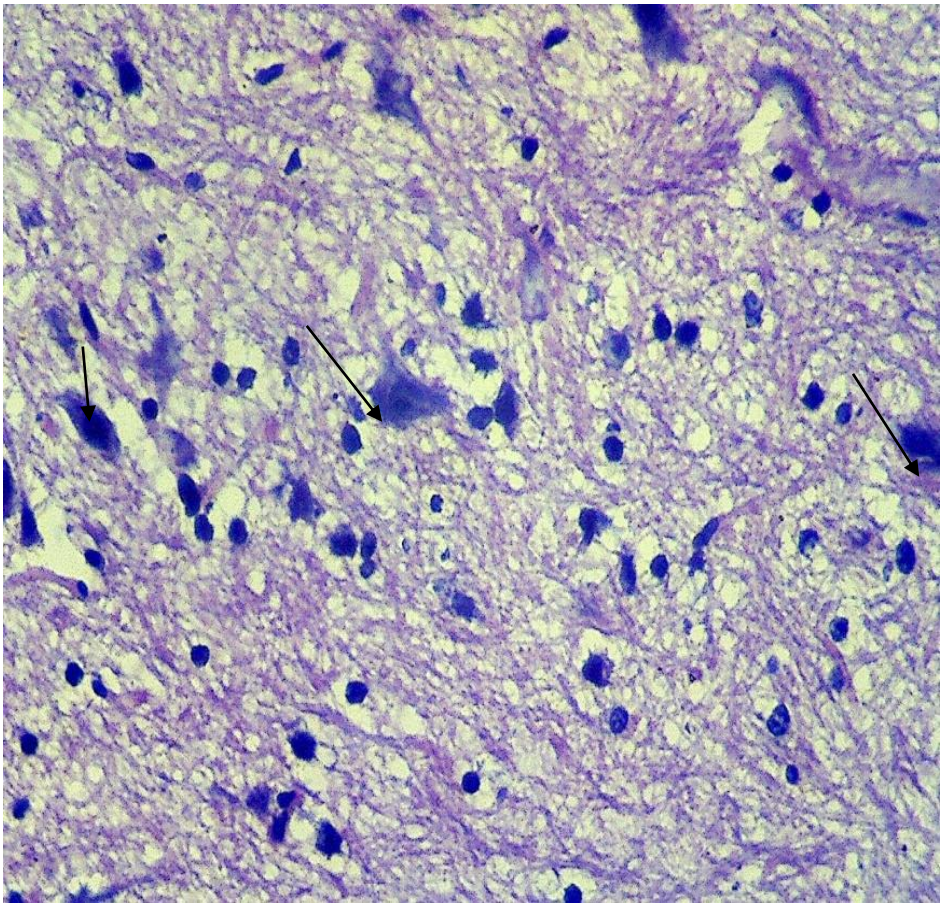


Fig:14. Brain of control group mouse, showing normal neuronal damage

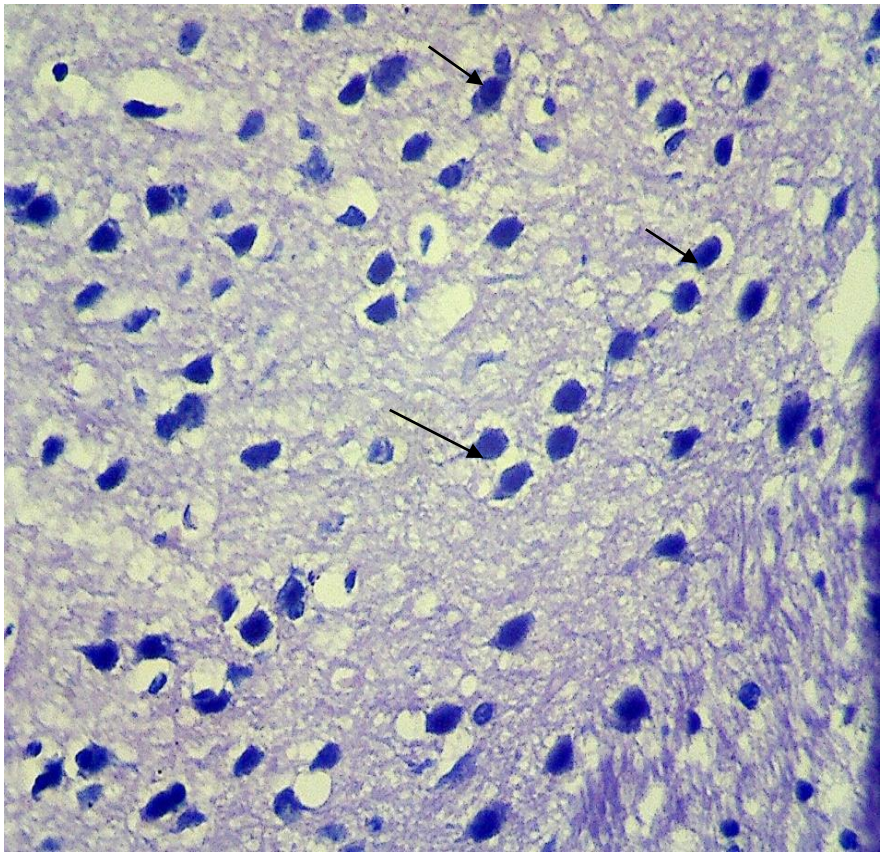


Fig:15. Brain of standard group mouse, showing normal neurons

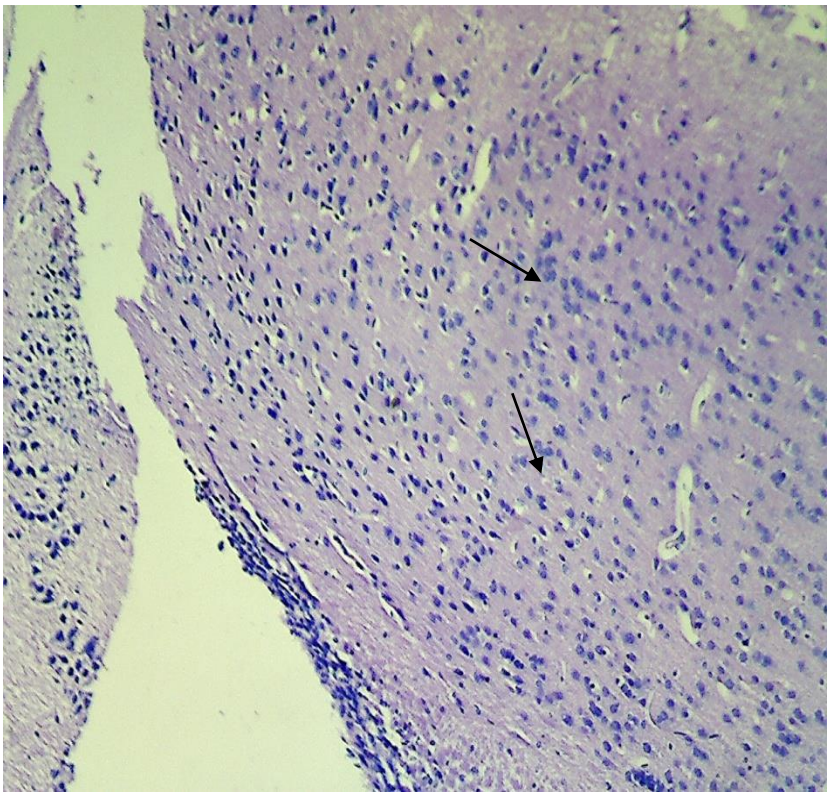


Fig:16. Brain of *Dolichandrone atrovirens* **leaves of chloroform extract 200mg (CEDA) group mouse, showing normal neurons**

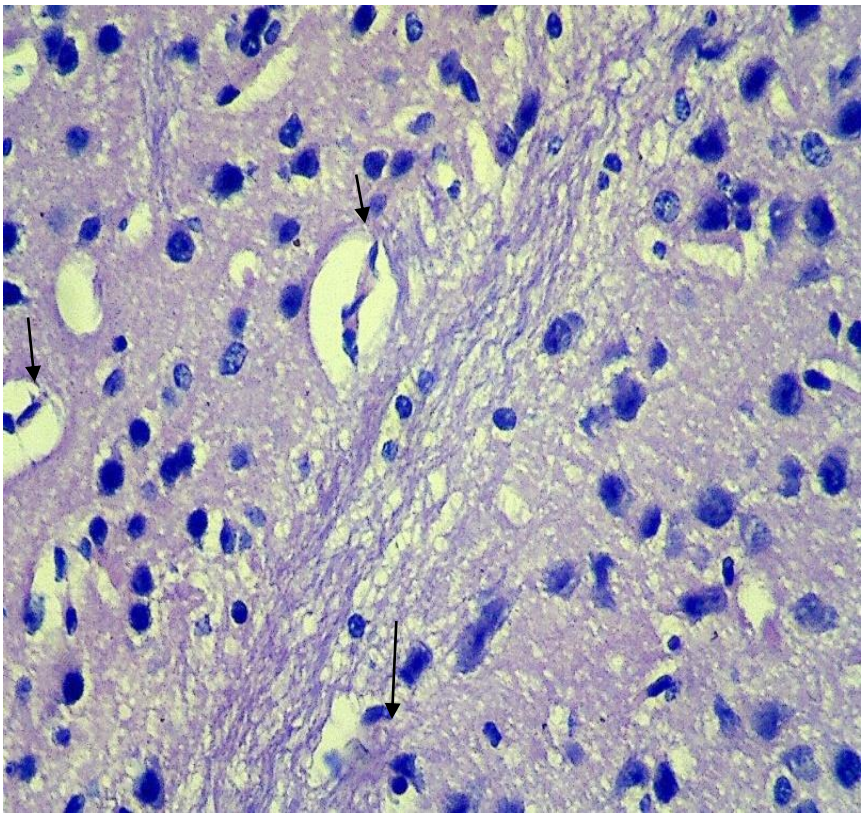


Fig:17. Brain of *Dolichandrone atrovirens* **leaves of chloroform extract (CEDA) 400mg group mouse, showing normal neurons**

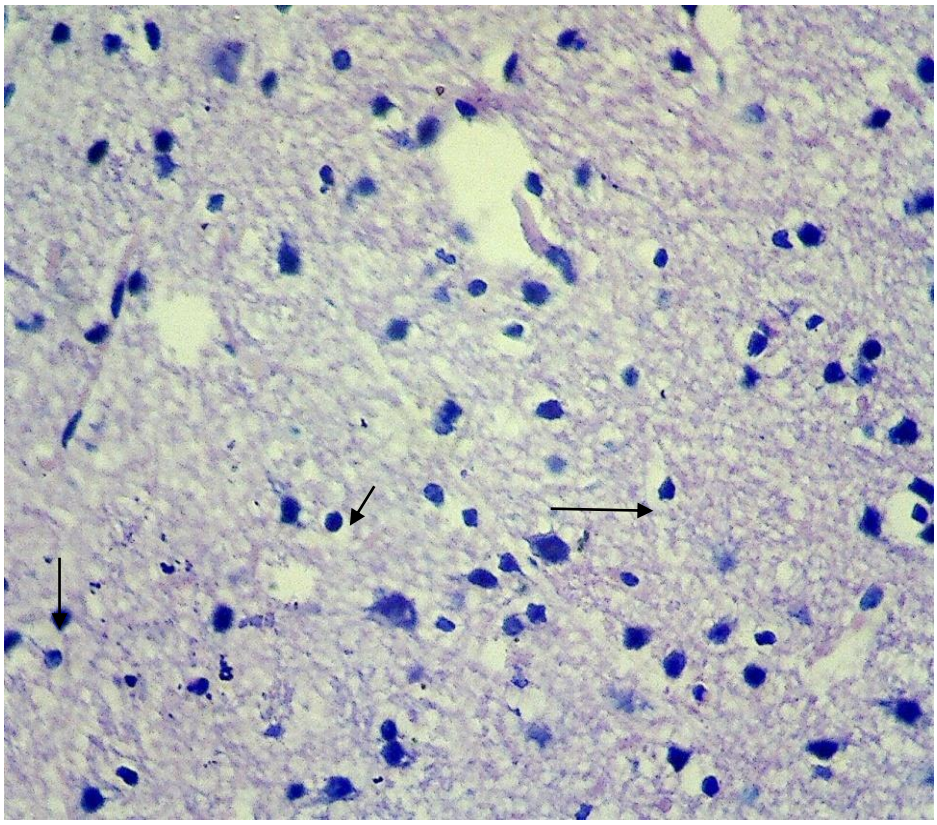


Fig:18. Brain of *Dolichandrone atrovirens* **leaves of ethanolic extract (EEDA) 200mg group mouse, showing normal neurons**

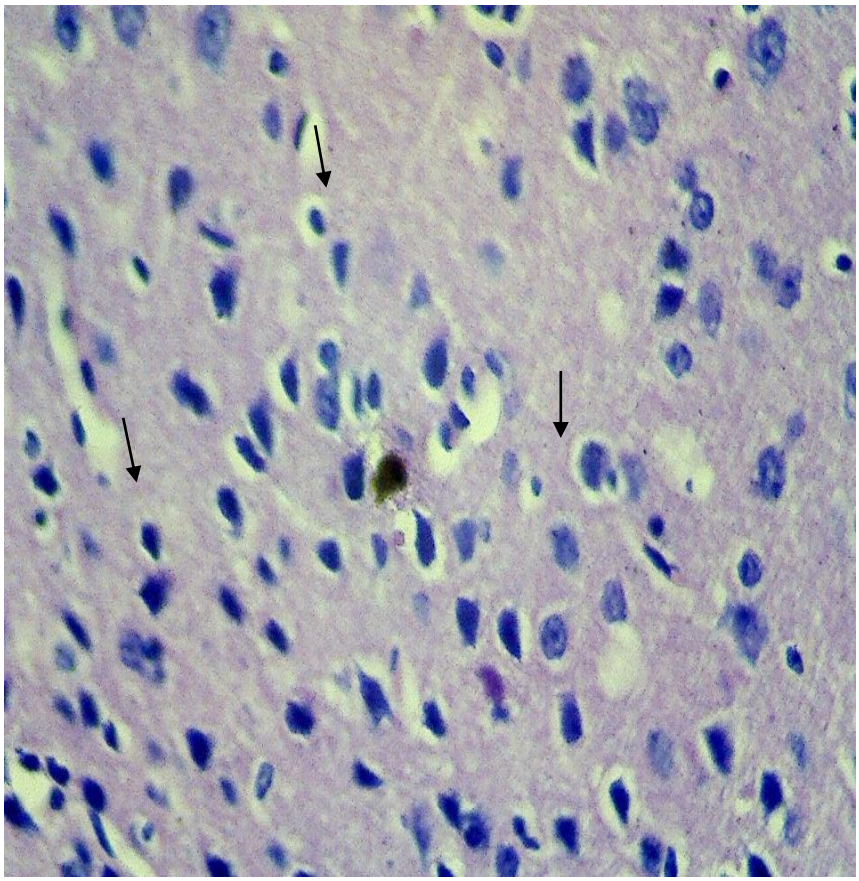


Fig:19. Brain of *Dolichandrone atrovirens* **leaves of ethanolic extract (EEDA) 400mg group mouse, showing normal neurons**

CHAPTER VI

DISCUSSION

Discussion

The alternative system of medicines like Ayurvedic, siddha, unani and other tribal folklore medicines have significantly contributed to the health care of the population of India. Today these systems are not only complementary but also competitive in the treatment of various diseases. Plants have served as a good source of CNS affecting diseases such as alzhiemer's, Parkinsonism, anxiety, epilepsy. Several studies have been conducted an herbs under a multitude of ethanobotanical grounds. A large number of plants possessing CNS properties have been documented.

It is now becoming exceedingly apparent that the available psychotherapeutics drugs do not properly meet the therapeutics demands of a vast majority of patients with mental health problems, and that herbal remedies remain the ultimate therapeutic hope for many such patients in the world. However, till now very little attention has been paid to develop functionally active CNS active drugs from psychoactive plants.

In the present study, the chloroform and ethanolic extracts shows the prolongation of diazepam-induced sleeping time and decrease in onset of action by diazepam might be attributed to action of extracts on the central mechanisms involved in the regulation of sleep. So both the extracts of *DA* leaves possessed the significant ($P<0.001$) CNS depressant activity.

The chloroform and ethanolic extracts of *Dolichandrone atrovirens* of leaves, produced the central inhibitory effects in mice. The locomotor activity was dose - dependent decrease in the number of counts as registered on Actophotometer. Thus the decrease in spontaneous motor activity indicates the extracts of *DA* having significant ($P<0.001$) CNS depressant property.

The lack of co-ordination in the rota rod test is characteristic of a drug that reduces the central nervous activity, such as neuroleptics, anxiolytics, sedatives and hypnotics. The rotating bar is useful to detect the muscle relaxant activity of test compound. The normal animals can be kept for a long period of time on the swivel bar. The ethanolic extract of both dose levels shows significant ($P<0.001$) changes in the

motor activity compare to chloroform extract. That is decrease in the motor co-ordination, results the neuromuscular blocking property. Preliminary phytochemical screening reveals that the presence of glycosides, carbohydrates, flavonoids, tannins and proteins in the plant extracts. Therefore, the observed skeletal muscle relaxant activity may be attributed to these compounds.[57]

The hole board test is useful for modeling anxiety in animals. This test the head dipping behavior was sensitive to changes in the emotional state of the animal, this extracts of *DA* significantly (P<0.001) increased the head dipping behaviors compare to control. This results shows both the extracts of *DA* having anxiolytic property[58]. The chloroform and ethanolic extracts at both the dose levels inhibited the maximal electroshock induced convulsions. This may also suggest that both the extracts having significantly (P<0.001) anti convulsant effect. The low dose of chloroform extracts of *DA* produced less effect, compare to ethanolic extract of plant.

In the light / dark test, anxiety is generated by the conflict between the tendency to explore and the initial tendency to avoid the unfamiliar and can be evaluated according to the number of transitions into and the time spent in the light chamber. Where in increase in these parameters is considered to reflect anxiolytic-like properties. This *DA* extracts shows the increased time spent in the light chamber, suggesting significant (P<0.001) anxiolytic action .The pharmacological profile of the present investigation of the chloroform and ethanolic extracts of *Dolichandrone atrovirens* was similar to that of benzodiazepines derivatives, it is also possible that they might interact with benzodiazepine receptor located adjacent to the GABA receptor [59].

The histological report in the brain of mice, does not shows any neuronal injury and glial proliferation.Therefore, the use of chloroform and ethanolic extracts of *Dolichandrone atrovirens leaves* in folkloric medicine may be presence of phytochemical constituents such as glycosides, carbohydrates, flavonoids, tannins and proteins in the plant extracts, for its CNS action.

CHAPTER VII
CONCLUSION

CONCLUSION

In the conclusion, the Chloroform and ethanolic extract of *Dolichandrone atrovirens* leaves extracts potentiated the diazepam – induced sleep and they decreased the spontaneous motor activity, indicating a central depressant effect. Both the extracts of *DA* reduced motor co-ordination in mice.This motor in coordination helped to concluded the extracts having neuromuscular blocking activity. Further the extracts reduced the duration of extension phase of MES .Finally the study shows the marked effect on anxiety behavioural parameters on exposure to light /dark test and in the hole board test in mice. The histopathological studies also reveals the neurological activity. The results obtained from these experimental models clearly confirmed the chloroform and ethanolic extract of leaves of *Dolichandrone atrovirens* possessed CNS depressant activity, anxiolytic activity and anti- convulsant activity.

Therefore further studies will be focused on the neurobiological mechanisms of action and possible interactions of *Dolichandrone atrovirens* with the neurotransmitters and the active compound responsible for the observed central effects has to be isolated and identified.

CHAPTER VIII

REFERENCES

REFERENCES

1. Farnsworth, Norman R. "Screening plants for new medicines." Biodiversity 1 (1988): 83-97.
2. Apata, L., Practice of herbalism in Nigeria ,university of life press, Nigeria,(1979).
3. Cragg , G.M. and Newman ,D.J.,J.Ethanopharmacology.,100,72-79,(2005).
4. https://en.wikipedia.org/wiki/World Health Organization.
5. Bassam Abdul Rasool Hassan, Medicinal Plants (Importance and Uses)" Pharmaceutica Analytica Acta 2012, 3:10
6. Nearing M, The Green Pharmacy Herbal Medicines In Modern Usage. IDRC Rep.,1985;14(1):10-14.
7. Singh R.. Medicinal Plants: A Review. Journal of Plant Sciences. Special Issue: Medicinal Plants. Vol. 3, No. 1-1, 2015, pp. 50-55
8. https://en.wikipedia.org/wiki/neuropharmacology
9. Fiala, john C., Joseph Spacek, and Kristen M.Harris."Dentritic spine pathology: Cause or consequence of neurological disorders?." Brain research reviews 39.1(2002):29-54
10. www.psychguides.com
11. Perry EK, Curtis M, Dick DJ, et al. Cholinergic correlates of cognitive impairment in parkinson's disease: comparison with Alzheimers disease. J. Neurol Neurosurg Psychiat 1985;48:413-421.
12. Tiraboschi P, Hansen LA, Alford M, et al. Cholinergic dysfunction in diseases with lewy bodies. Neurology 2000;54:407-411.
13. Ann S. Morrison, Constantine Lyketsos, "The Pathophysiology Of Alzheimer's Disease And Directions In Treatment" Advanced Studies In Nursing,2005, Vol 3, No 8, 256-270.
14. www.healthline.com/health/seizures

15. Engelborghs,S., Hooge R.D, and .De Deyn P.P.” Pathophysiology Of Epilepsy.” Acta neurologica belgica 100.4(2000):201-213.

16. www.epilepsycolorado.org.

17. www.epilepsy.com

18. Kavimani, S., K. Saminathan, and R. Senthil Kumar. "Antioxidant and free radical scavenging activities of Dolichandrone atrovirens using various in vitro assay models." *International Journal of Phytopharmacology* 5.4 (2014): 293-300.

19. Kavimani, S., K. Saminathan, and R. Senthil Kumar. "In vitro antidiabetic activity of *Dolichandrone atrovirens* an Indian medicinal plant." International Journal of Pharmacotherapy 4.3 (2014): 107-113.

20. kayarohanam, S., and kavimani S. "quantitative phytochemical and gc-ms analysis of leaf and bark extract of *dolichandrone atrovirens."* international reaserch journal of pharmacy 6.4 (2015): 219-222

21. Saminathan kayarohanam, kavimani. S, “ Acute And Sub-Acute toxicity study of aqueous methanolic leaf and bark extract of *dolichandrone atrovirens”* International Journal of Pharmacy and Pharmaceutical sciences ISSN- 0975-1491 vol 7, issue 6, 2015

22. Natarajan P, Arunpandian R, evaluation of anti-cancer activity *dolichandrone atrovirens* in experimental induced animal model” indo American Journal of Pharmaceutical Research, 2015:5(07)

23. Saminathan Kayarohanam and kavimani S, Preliminary Phytochemical Screening and HPLC analysis of bark and leaf extract of *dolichandrone atrovirens,* World journal of pharmaceutical sciences

24. Saminathan Kayarohanam, kavimani. S, Histopathological studies of aqueous methanolic leaf and bark extract of *dolichandrone atrovirens*, World Journal of Pharmacy and Pharmaceutical Sciences ,volume 4, issue 07, 733-741.

25. Kumaresan P. Thirupathy, Asish Tulshkar1, Vijaya C, Neuropharmacological activity of *Lippia nodifl ora* Linn. Pharmacognosy Research | July 2011 | Vol 3 | Issue 3 Issue 12

26. Hitender Sharma, Munish Garg, Neuropharmacological activities of *Taxus wallichiana* bark in Swiss albino mice, Indian Journal of Pharmacology | June 2015 | Vol 47 | Issue 3 299

27. Bhosale, Uma A., et al. "Study of central nervous system depressant and behavioral activity of an ethanol extract of *Achyranthes aspera* (Agadha) in different animal models." International Journal of Applied and Basic Medical Research 1.2 (2011): 104.

28. Pal, Dilipkumar, et al. "CNS depressant activities of roots of *Coccos nucifera* in mice." Acta Pol Pharm 68.2 (2011): 249-54.

29. Gurunath, Hogade Maheshwar, Vanita Chature, and Digge Vaijinath. "Anticonvulsant activity of whole plant of *Solanum surattense* Burm against MES and PTZ induced seizures in rats." International Research Journal of Pharmacy 2.1 (2011): 275-8.

30. Bose, Utpal, et al. "Anti-inflammatory and Neuropharmacological activities of *Caesalpinia pulcherrima* bark." Research Journal of Pharmaceutical, Biological and Chemical Sciences 2.1 (2011): 77-84.

31. Perez G.R.M., et al. "Neuropharmacological Activity Of *Solanum nigrum* Fruit." Journal Of Ethnopharmacology 62.1 (1998): 43-48.

32. Khalid, Mohammed Saifuddin, Shahid.Abrar, and Hakeem. Uddin. Khan. "anti-anxiety effect on ethanolic extracts of *agave americana* linn." Asian Journal of Pharmacy and Clinical research 6.suppl 1 (2013): 43-48.

33. Jivad, Nahid, and Zahra rabiei. "A Review Study On Medicinal Plants Used In The Treatment Of Learning And Memory Impairments." Asian Pacific Journal Of Tropical Biomedicine 4.10 (2014): 780-789.

34. Tomar, Sunil K., Gaurav Sharma, and Mathew George. "Skeletal Muscle Relaxant Activity of chloroform extract of *phyllostachys bambusoides* on wistar rats." (2012).

35. Pitchaiah, Gummalla, et al. "anxiolytic and anticonvulsant activity of methanolic extract of *allium cepa* linn (onion) bulbs in swiss albino mice." Journal Of Pharmacognosy And Phytochemistry 4.3 (2015)

36. Mohamed Nadjmouddine,Yahya Cherrah ,Katim,Khalid Tahghzouti,"Evaluation Of Anxiolytic Activity of methanolic extract of *urtica urensin* a mice model. behavioral and brain functions.2015.

37. Dr. Deepa. Halemani, Dr. Geetha. M, Dr. Shashikala G. H, Evaluation Of Anti Anxiety Activity of methanol extract of *aegle marmelos* (bael fruit tree) leaves in rats, IOSR Journal Of Dental And Medical Sciences (IOSR-JDMS), volume 14, issue 9 ver. vi (sep. 2015), pp 01-05

38. Sutar, Ravindra C., Sanjay B. Kasture, And V. K. Kalaichelvan. "evaluation of anticonvulsant activity of leaf extracts of *holoptelea integrifolia*(roxb.) planch in experimental animals." International Journal Of Pharmacy & Pharmaceutical Sciences 6 (2014): 308-311.

39. Uabundit, Nongnut, et al. "Cognitive Enhancement And Neuroprotective effects of *bacopa monnieri* in alzheimer's disease model." Journal Of Ethnopharmacology 127.1 (2010): 26-31.

40. Haleagrahara, Nagaraja, And Kumar Ponnusamy. "Neuroprotective Effect Of *Centella asiatica* extract (cae) on experimentally induced parkinsonism in aged Sprague-dawley rats." the journal of toxicological sciences 35.1 (2010): 41-47.

41. Bastianetto, Stéphane, et al. "Neuroprotective effects of green and black teas and their catechin gallate esters against β-amyloid-induced toxicity." European Journal of Neuroscience 23.1 (2006): 55-64.

42. Yu, Man-Shan,"Neuroprotective effects of anti-aging oriental medicine *Lycium barbarum* against β-amyloid peptide neurotoxicity." Experimental gerontology 40.8 (2005): 716-727.

43. Indiabiodiversity.org/species/show/229569

44. www.theplantlist.org/tpl/record/kew-320740

45. www.flowersofindia.net/catalog/slides/wavy%20trumpet%20flower.Html

46. El-Khatib AA. Environ pollut.2003;124(3):419-428

47. Sharma K,Dutta N,Pattanaik AK,Hasan QZ.Trop Anim Health prod.2003 Apr;35(2):131-145.

48. Pulok.K.Mukherjee,Quality control of herbal drugs,1st edition business horizons publishers,2002 India.

49. https://en.wikipedia.org/wiki/Acute -toxicity

50. Draft OECD guideline for the testing of chemicals, test guideline 452: chronic toxicity studies, november 2008.

51. Parasuraman, s. "toxicological screening." Journal Of Pharmacology And Pharmacotherapeutics 2.2 (2011): 74.

52. Tirumalasetti J, Patel M, Shaikh U, Harini K, Shankar J. evaluation of skeletal muscle relaxant activity of aqueous extract of *nerium oleander* flowers in albino rats. Indian Journal Of Pharmacology 2015;47:409-13

53. "Sedative, Anxiolytic And Anticonvulsant effects of different extracts from the leaves of ipomoea carnea in experimental animals" int. j. drug dev. & res., april-june 2013, 5(2): 232-243.

54. Ravindra C. Sutar, Sanjay B. Kasture, V. K. Kalaichelvan, Evaluation Of Anticonvulsant activity of leaf extracts of *holoptelea integrifolia* (roxb.) planT in experimental animals, International Journal Of Pharmacy And Pharmaceutical Sciences, vol 6, issue 4, 2014.

55. Ramanathan M, Sivakumar S, .Anandvijayakumar PR, Saravanababu C, Rathinavel P, Pandian, "Neuroprotective evaluation of standardized extract of *Centella asciatica* in monosodium glutamate treated rats, Indian Journal Of Experimental Biology,Vol.45,May 2007,PP.425-431

56. Parihar M.S, Taruna Hemnani," Phenolic antioxidants attenuate hippocampal neuronal cell damage against kainic acid induced excittotoxicity.

57. Sunil k tomar, Gaurav sharma, Mathew george ,skeletal muscle relaxant activity of chloroform extract of *phyllostachys bambusoides* on wistar rats," international journal of pharmacy and pharmaceutical sciences" vol 4, suppl 3, 2012.

58. Mohammed saifuddin khalid, Shahid abrar, hakeemuddin khan, " anti-anxiety effect on ethanolic extracts of *agave americana* linn, " asian journal of pharmaceutical and clinical research vol 6, suppl 1, 2013

59. Thiago henrique costa marques1, Maria leonildes boavista gomes castelo branco marques1, douglas dos santos lima2, hálmisson d'árley santos siqueira, "evaluation of the neuropharmacological properties of nerol in mice", world journal of neuroscience, 2013, 3, 32-38.